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THE TOXICITY OF VARIOUS IODINE SOLUTIONS*

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IN 1923 Plummer[1] definitely established the use of iodine in the pre-operative treatment of thyrotoxicosis. He used Lugol's solution, 5 per cent iodine, and 10 per cent potassium iodide, but since then numerous other iodine solutions have been prepared for similar use. We have made a study of the relative toxicity of some of the iodine solutions used clinically, such as Lugol's solution, 10 per cent sodium iodide, 10 per cent potassium iodide, Amend's solution and others, which will be mentioned later. Amend's solution is essentially a mixture of sodium iodide and iodine, the "free" iodine being adsorbed to a protein precipitable with tungstic acid. It was decided to compare the relative toxicity of these solutions by the blood pressure method, the intravenous "emesis" point, and the blood iodine tolerance test, and by administering them to human patients known to be sensitive to iodine.

Blood Pressure Method.—Dogs anesthetized with basal ether anesthesia were used. A continuous record of blood pressure and respiration was made. The iodine solutions were injected intravenously during a period of ten to fifteen seconds. The blood pressure was always allowed to return to the control level before the injection of a solution. Doses of each solution equivalent in regard to iodine content were always used. The dogs weighed approximately 10 kg.

The results on the most interesting solutions of iodine are best compared by inspecting the composite curves of the results on 5 dogs in Chart 1. The curves show the average blood pressure depressing effect of 0.1, 0.2, 0.3, and 0.4 gm. of iodine in the various solutions. The composite results on the other solutions are shown in Table I in more detail.

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TABLE I

COMPARATIVE AVERAGE EFFECT OF VARIOUS IODINE SOLUTIONS ON BLOOD PRESSURE*

SOLUTION	GM. I	MG. PER KG.	AVERAGE B.P. CHANGE IN MM.	REACTION TIME
Amend's	0.1	9-11	3	Immediate reaction returning to normal in 30 sec.
Lugol's	0.1	9-11	16	Immediate reaction returning to normal in 30 sec. to 60 sec.
10% KI	0.1	9-11	13	Immediate reaction returning to normal in 60 sec.
10% NaI	0.1	9-11	2	Immediate reaction returning to normal in 30 sec.
Amend's	0.195	18-22	2	Immediate reaction returning to normal in 10 sec. to 30 sec.
Lugol's	0.195	18-22	22	Immediate reaction returning to normal in 60 sec.
10% KI†	0.195	18-22	9	Immediate reaction returning to normal in 30 sec. to 60 sec.
10% NaI	0.195	18-22	4	Immediate reaction returning to normal in 10 sec.
Amend's	0.390	36-44	12	Gradual slow decrease returning to normal in 2 to 3 min.
Lugol's	0.390	36-44	2 cases—fatal	Immediate reaction
10% NaI	0.390	36-44	4	Immediate reaction returning to normal in 10 sec.
Amend's	0.47	52	8	Gradual slow decrease returning to normal in 3 min.
Amend's	0.1	3-7	2	Immediate reaction returning to normal in 30 sec.
K Lugol's	0.1	3-7	22	Immediate reaction returning to normal in 1 min. 30 sec.
Na Lugol's	0.1	3-7	14	Immediate reaction returning to normal in 1 min.
Na Lugol's plus 1% gelatin	0.1	3-7	5	Immediate reaction returning to normal in 30 sec.
Amend's	0.2	6-14	2	Immediate reaction returning to normal in 1 min.
K Lugol's	0.2	6-14	57	Immediate reaction returning to normal in 1 min. 30 sec.
Na Lugol's	0.2	6-14	20	Immediate reaction returning to normal in 1 min.
Na Lugol's plus 1% gelatin	0.2	6-14	8	Immediate reaction returning to normal in 45 sec.
Amend's	0.3	12-28	2	Immediate reaction returning to normal in 1 min.
K Lugol's	0.3	12-28	124	Gradual reaction returning to normal in 5 min.
Na Lugol's	0.3	12-28	34	Immediate reaction returning to normal in 1 min. 30 sec.
Na Lugol's plus 1% gelatin	0.3	12-28	22	Immediate reaction returning to normal in 1 min.
Amend's	0.4	24-56	6	Immediate reaction returning to normal in 1 min. (only case—40 mg. per kg.)

*Five dogs were used ranging from 9 to 11 kg.; ether anesthesia used in all cases.
†In two cases this dosage caused death of the animals.

When 0.1 gm. of iodine was injected in the form of the various solutions, Amend's solution depressed blood pressure four times less than 10 per cent potassium iodide, five times less than Lugol's solution, while 10 per cent sodium iodide had the same effect as Amend's solution.

When 0.2 gm. of iodine was injected, Lugol's solution depressed blood pressure eleven times more than Amend's solution, and 10 per cent sodium iodide two times more. Two dogs died after the 0.2 gm. of 10 per cent potassium iodide.

When 0.4 gm. of iodine as Lugol's solution was injected, it proved fatal in 2 of 5 dogs. Amend's solution and 10 per cent sodium iodide had a relatively slight effect.

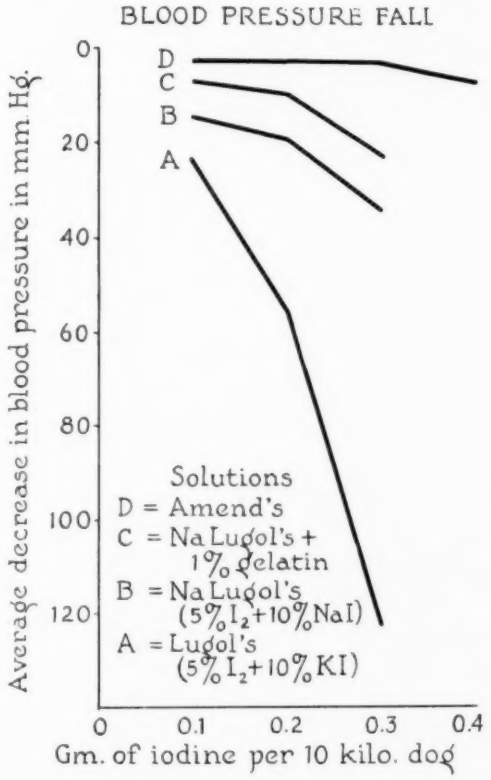

Chart 1.

Since Amend's solution is essentially a mixture of sodium iodide and iodine, the iodine being bound to a proteinlike substance, the toxicity of Amend's solution and a solution of 10 per cent sodium iodide and 5 per cent iodine were compared by the blood pressure method. The sodium-Lugol's solution was prepared and injected in equivalent iodine doses. The composite results may be compared by inspecting curves B and D in Chart 1. In doses of the two solutions of from 0.1 to 0.3 gm. of iodine, Amend's solution is less toxic than sodium-Lugol's solution, although the latter is considerably less toxic than potassium-Lugol's solution. This showed that the proteinlike substance in Amend's solution actually buffers the free iodine.

We then tried the effect of adding proteins to the sodium-Lugol's solution. We used 1 per cent gelatin, 0.5 and 1 per cent egg albumen, and 1 and 2 per cent gastric mucin. All the solutions proved to be less toxic by the blood pressure depressing test than sodium-Lugol's solution alone. The least toxic solution was sodium-Lugol's plus 1 per cent gelatin. The comparative effects of Amend's and sodium-gelatin-Lugol's solution are shown in Chart 1. The results show that protein substances can serve as a buffer for free iodine and reduce the toxicity of iodine solutions, such as sodium-Lugol's solution.

TABLE II

SUBJECT	AMOUNT IODINE 100 MG. I	BLOOD IODINE PER 100 C.C.		URINE IODINE TOTAL PER 24 HR.		% IODINE ELIMINATED IN URINE
		CONTROL	PEAK	CONTROL	POST IODINE	
J	Amend's	1.4	15.5	45.0	763.0	0.7
	Lugol's	5.6	8.4	180.0	785.0	0.7
M	A.	1.4	7.0	53.0	3603.0	3.6
	L.	5.6	8.4	168.0	1220.0	1.2
M	A.	0.70	8.4	20.0	8265.0	8.2
	L.	1.4	9.8	108.0	11426.0	11.4
R	A.	5.6	12.7	322.0	1161.0	1.2
	L.	4.2	11.3	193.0	835.0	0.8
B	A.	2.1	21.1	66.0	3993.0	3.9
	L.	2.1	9.8	94.0	2536.0	2.5
H	A.	2.8	11.3	85.0	584.0	0.5
	L.	2.8	11.3	46.0	695.0	0.7
J	A.	1.4	12.7	124.0	911.0	0.9
	L.	4.2	12.7	183.0	920.0	0.9
A	A.	1.4	8.4	146.0	7718.0	7.7
	L.	2.1	8.4	76.0	4104.0	4.1
Average	A.	2.1	12.1	108.0	3375.0	3.4
	L.	3.3	10.0	131.0	2815.0	2.8
Dog 1	A.	2.1	12.0	14.5	765.0	0.7
	L.	2.1	5.6	9.8	197.0	0.2
Dog 2	A.	2.8	6.4			
	L.	5.6	13.1			
Dog 3	A.	3.5	23.9	11.0	886.0	0.9
	L.	5.6	16.8	13.2	246.0	0.2
Dog 4	A.	2.9	26.0	8.8	322.0	0.3
	L.	5.6	28.1	6.7	152.0	0.2
Average	A.	2.8	17.0	11.4	658.0	0.6
	L.	4.7	15.9	9.9	198.0	0.2

Emesis Point.—Experience has taught that dogs with food in their stomachs are quite sensitive in regard to the production of emesis when certain toxic substances are injected intravenously. We used this as a test of the relative toxicity of Amend's and Lugol's solutions when given intravenously to unanesthetized dogs. The dogs were fed a meal, and the least amount of the two solutions required to cause emesis was determined. Five of 6 fed dogs of approximately the same body weight (10 kg.) vomited after the intravenous injection of 100 mg. of iodine in the form of Lugol's solution. Vomiting did not occur with smaller doses. One hundred milligrams of iodine as Amend's solution had no effect. One hundred and fifty milligrams of Amend's solution caused nausea (salivation), slight retching, but no vomiting, in 6 dogs.

In 2 out of 4 dogs 50 mg. of iodine as Lugol's solution was given by stomach tube and the tube washed with 100 c.c. of water; salivation and apparent nausea, but no vomiting, occurred (stomach empty). When 50 mg. of iodine as Amend's solution were given similarly, salivation and other objective symptoms did not result. This difference can be interpreted as showing that the Lugol's solution irritated the stomach and duodenum more than the Amend's solution, the "free" iodine of which is adsorbed to protein.

Blood Iodine Curves.—Iodine is normally present in the blood in an average concentration of 4 gamma per cent.[2] Numerous investigators have shown that for a period of several hours after the ingestion of iodine, the blood iodine rises to a maximum and then falls, producing a blood iodine curve. Accordingly, the blood iodine curves of 8 normal human subjects after the ingestion of equivalent iodine doses of Amend's and Lugol's solutions were determined. The iodine tolerance method of Perkins and his co-workers[3] was used. The results for the human and animal studies are given in Table II. In man, the blood iodine increased from 2 to 3 gamma per cent to 12 gamma per cent, and the peak was reached in the second hour after ingestion. In dogs the blood iodine curves for both Amend's and Lugol's solutions show similar but greater changes. The blood iodine increased from 3 to 5 gamma per cent to 15 gamma per cent. However, when Lugol's solution was taken, the peak in blood iodine occurred in one-half an hour, whereas with Amend's solution it occurred in the second hour. We have no explanation for this phenomenon. The difference between the average control and maximum values for Amend's solution and Lugol's solution is well within the experimental error of the blood iodine method.

The amount of iodine eliminated in the urine of human beings, when either Amend's or Lugol's solution was taken, also shows a close correspondence (Table II). The small amount eliminated can be explained by the fact that the iodine method used gives results which are 8 to 10 per cent lower than those obtained by the earlier methods.

Rate of Absorption.—The rate of absorption from the gastrointestinal tract of human subjects was determined by the time of occurrence of the peaks in the blood iodine curves, and also by the time of appearance of iodine in the saliva after receiving 50 mg. of iodine solution by Rhefuss' stomach tube. In all cases, when either Amend's or Lugol's solution was ingested, the iodine was detected in the saliva within fifty to sixty minutes. The salivary glands were not stimulated by any such mechanical means as paraffin chewing, which accounts for the delayed appearance in the saliva.

Uptake of Iodine by the Thyroid.—We have followed the method of Van Dyke[5] in this work. Eight dogs, weighing approximately 6 kg. each, were used and anesthetized with nembutal (sodium pentobarbital). After careful exposure of the lobes of the thyroid, a control sample consisting of the lower pole of either the right or left lobe was removed. In all cases it was found that the difference in control iodine content between right and left lobes was insignificant. Then, either Lugol's or Amend's solution in 50 mg. iodine doses was injected into the femoral vein. After thirty minutes a suitable sample of the gland was

removed. The remaining iodine solution was then injected, and after thirty minutes the rest of the thyroid tissue was excised. All samples were weighed fresh, and the iodine concentration determined immediately by Stevens' method.[3] Table III gives the results obtained from analyzing portions of the thyroid gland before and after iodine injection. Although the iodine content per gram of the control lobe was found to be very small, the iodine concentration increased several hundred per cent after injection of an iodine solution. It mattered very little whether Amend's or Lugol's solution was used, since, in either case, the iodine was utilized to approximately the same extent by the thyroid tissue.

TABLE III

UPTAKE OF VARIOUS IODINE SOLUTIONS BY THE THYROID GLAND

EXPER. NO.	WT. OF DOG (KG.)	50 MG. I INJECTED	WT. OF CONTROL LOBE FRESH (MG.)	GAMMA I PER MG. OF CONTROL LOBE	MG. I PER GM. CONTROL LOBE	WT. OF IODIZED LOBE FRESH (MG.)	GAMMA I PER MG. OF IODIZED LOBE	MG. I PER GM. OF IODIZED LOBE	MG. I INCREASE PER GM. OF GLAND
1	5	Amend's	140	0.005	0.005	120	0.51	0.510	0.505
		Lugol's				81	0.50	0.500	0.495
2	5	Amend's	250	0.0028	0.0028	180	0.24	0.240	0.237
		Lugol's				290	0.14	0.140	0.137
3	6	Amend's	230	0.003	0.003	670	0.08	0.08	0.077
		Lugol's				600	0.09	0.09	0.087
4	6	Amend's	130	0.005	0.005	440	0.13	0.13	0.125
		Lugol's				580	0.13	0.13	0.125
5	6	Amend's	150	0.004	0.004	260	0.12	0.12	0.116
		Lugol's				250	0.12	0.12	0.116
6	6	Amend's	500	0.0014	0.0014	390	0.15	0.15	0.14
		Lugol's				860	0.10	0.10	0.09
7	6	Amend's	210	0.003	0.003	420	0.10	0.10	0.097
		Lugol's				340	0.11	0.11	0.107
8	6	Amend's	135	0.005	0.005	325	0.16	0.16	0.155
		Lugol's				505	0.13	0.13	0.125

Iodine-Sensitive Human Subjects.—In this study we were able to obtain only 3 persons who manifested hypersensitivity to iodine. Two of these subjects manifested the most usual type of iodine hypersensitivity, namely, sialorrhea. One hundred milligrams of iodine as Amend's solution when given orally resulted in excessive salivation and nausea which lasted for thirty-six hours; 20 mg. of iodine as Lugol's solution gave similar toxic effects.

The third subject was extremely hypersensitive to iodine when taken orally or applied to the skin. When Amend's and Lugol's solutions in equivalent iodine doses were applied to the inner aspect of the forearm, the area painted with Lugol's solution showed a definite irritation the next day, and in three days a marked dermatitis with redness, edema, and small papules. The Amend's solution produced no reaction.

DISCUSSION

Essentially there are two groups of iodine solutions: those that contain the sodium cation and those with the potassium cation. That this is an important

factor in determining the toxicity of an iodine solution has been fairly well established. It is pharmacologically known that in large doses the potassium ion is more toxic than the sodium ion. Further, Osborne[4] has reported that when potassium iodide is ingested, an average of 15 per cent of the iodine is present in the plasma in combination with protein and the rest as sodium iodide, there being no evidence that potassium iodide is absorbed as such. It was found that potassum iodide is not as well tolerated as sodium iodide, and that more protein iodide is formed by iodine-hypersensitive patients than in the noniodine-sensitive patients. However, we are not in a position to say that more protein iodide is formed with those iodine solutions containing the potassium ion than those with the sodium ion; this would constitute a major investigation.

In an attempt to decrease the toxicity of iodine solutions containing free iodine, various organic substances have been added which physically combine with the free iodine. Thus, the decreased toxicity of Amend's solution in comparison with other iodine solutions can be explained by the presence of the proteinlike substance which acts as a "buffering agent" for the iodine. However, there is no reason to believe that the free or loosely-linked iodine in Amend's solution is absorbed as such. More likely, the loose linkage between the free iodine and the proteinlike substance would be broken, and the iodine converted primarily into sodium iodide and absorbed as the iodide. That sodium iodide is practically as toxic as Amend's solution and much less toxic than Lugol's solution and potassium iodide is borne out by our blood pressure experiments. However, if Lugol's solution is administered in milk, the milk would buffer the free iodine, thus greatly alleviating the irritating effects of this solution when taken orally. Hence, it may be said, that if Amend's and Lugol's solutions were given in milk, there should be no difference between the doses of iodine required to excite the iodine-sensitive patient. What effect the formation of protein iodide has in reference to iodine sensitivity should be investigated thoroughly. It would also be very interesting to know whether the formation of protein iodide represents the essential feature of the therapeutic value of iodine or the toxic manifestations of iodine, or both.

SUMMARY AND CONCLUSIONS

We have investigated the relative toxicity of various iodine solutions in reference to blood pressure depression, to the emesis point when injected intravenously or when taken orally, to the blood iodine curve, and to their effect in iodine-sensitive patients.

The relative toxicity of an iodine solution administered orally, intravenously, or percutaneously, appears to be related to the presence of the sodium or potassium ion in the solution, to the presence of a buffering agent to neutralize free iodine if the latter is present, and possibly to the amount of protein iodide formed during absorption of orally administered iodine.

Amend's solution, which according to our analysis is essentially a solution of sodium iodide containing free iodine adsorbed to a protein, and sodium iodide are less toxic when given intravenously, are less irritating to the stomach when taken orally, and are better tolerated by iodine-sensitive subjects than Lugol's

solution in iodine-equivalent doses. According to the results of the blood iodine curve and the uptake of iodine by the thyroid, Amend's solution and sodium iodide should be as effective as Lugol's solution in the treatment of hyperthyroidism when given orally in iodine-equivalent doses.

REFERENCES

1. Plummer, H. S.: Results of Administering Iodine to Patients Having Exophthalmic Goiter, J. A. M. A. **80**: 1955, 1923.
2. Stevens, C. D.: Determination of Iodine in Biological Materials, J. Lab. & Clin. Med. **22**: 1074, 1937.
3. Perkins, H. J., Brown, B. R., and Lang, J.: The Blood Iodine Content of Normal and Thyrotoxic Individuals. An Iodine Tolerance Test, Canad. M. A. J. **31**: 365, 1934.
4. Osborne, E. D.: Contributions to the Pharmacology and Therapeutics of Iodide, J. A. M. A. **79**: 615, 1922.
5. Van Dyke, H. B.: Iodine Compounds. Their Selective Absorption by the Hyperplastic Thyroid Gland of the Dog, Arch. Int. Med. **41**: 615, 1928.

THE CONCENTRATION OF VITAMIN C IN THE BLOOD DURING AND AFTER PREGNANCY*

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I. Theoretical Part

THE quantity of ascorbic acid excreted in the urine after a tolerance test of vitamin C is a widely used indicator of vitamin C deficiency in human beings. This method, generally regarded as the best, furnishes information concerning the state of repletion of the vitamin C reserves of the body. It is an open question, however, whether deficiency or absence of reserve vitamin C is an indication of pathologic hypovitaminosis or avitaminosis. In laboratory experiments with guinea pigs, for example, it could be clearly shown that the dose of vitamin C necessary for the maintenance of reserve in a state of repletion is almost ten times as great as the minimum protective dose. Guinea pigs given the minimal dose of vitamin C enjoyed full health and life, even though the vitamin C content of their tissues was minimal. Moreover, it was found that guinea pigs whose vitamin C reserve was brought to a maximum by providing them with a diet rich in vitamin C were just as susceptible to avitaminosis when transferred to a diet deficient in vitamin C as guinea pigs which had not previously been provided with a body store of vitamin C.[1] This finding is highly surprising. The observations are strictly applicable for the present only for the case of guinea pigs, but there is no valid reason for assuming that the relationship in human beings is essentially different. Pending further evidence, it seems fair to conclude that before the repletion test can be correctly used as an indicator of avitaminosis, proof must be advanced that it has a different significance in human beings than in guinea pigs. The experimental data now

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available support the conclusion that a heavy retention of vitamin C in the repletion test is evidence that the body vitamin C reserves are partially or totally exhausted but it is not by any means an indication of hypovitaminosis or avitaminosis.

Despite the low level of the reserve, vitamin C metabolism may proceed along normal lines. Every case of scurvy will, therefore, show a marked retention of fed vitamin C, but not every case of retention is an indication of scurvy or even of "prescurvy." The span between the protective and the storage dose is considerable. In statistical studies based on repletion tests the number of healthy individuals classed as deficient remains an unknown factor.

It has been pointed out that the experiments with guinea pigs show that the previous repletion of the body vitamin reserve does not confer a significant advantage. This does not mean that the minimum dose and no more should be administered, but it does suggest that the feeding of large vitamin C quantities is of doubtful utility. The possibility remains that pharmacologically vitamin C in large doses may be useful as a detoxicant; this aspect is not, however, related to the normal function of vitamin C in the body.

Recent experiments throw some further light on the foregoing findings. It has been shown that the behavior of vitamin C in the urinary excretion is that of a threshold substance.[2] Its serum concentration must be raised from a minimum level of 0.78 mg. per cent or from a medium level of 1.0 mg. per cent to a level of 1.4 mg. per cent before excretion can occur. The latter is the threshold saturation level, but 0.78 mg. per cent is still normal and is by no means an indication of scurvy.

It must further be remembered that the excretion of vitamin C in urine depends on several factors. The excretion is greater and the retention is less, for instance, in acid than in alkaline urine. The vitamin concentration of single urine samples is no longer regarded as significant. Even determinations carried out on a day urine may be misleading.

We believe that the surest method of determining an actual vitamin deficiency in contradistinction to a mere reserve deficiency is provided by assaying the concentration of vitamin C in the blood. It is known that the blood vitamin C level reflects the vitamin C balance of the organism.[3] Symptoms of scurvy are unfailingly accompanied by a low blood concentration of vitamin C. Recovery from the disease is accompanied by a corresponding increase in its blood concentration. Thus in the survey of the food distributed by the social service agencies we are able to show that the vegetable and fruit content of the diet in different districts is correlated with the concentration of vitamin C found in the blood. This latter averaged about 1 mg. per cent in our findings; the minimum was never less then 0.78 mg. per cent.

The available information of the behavior of vitamin C in the blood was found to be deficient in several respects. To fill this lack, some general experiments on vitamin C in blood were carried out before work on clinical aspects was undertaken. Vitamin C (reduced form) was determined in the blood according to the method of Farmer and Abt. In this method, as in most others, Tillman's reagent is used for titration. The validity of the values obtained with

this reagent in urine and other biological fluids has been questioned.[4] It must be shown, therefore, that in the method adopted it is actually only vitamin C which is titrated. It may be noted that Farmer and Abt[3] have shown that in scurvy the reducing values of the blood are very low, but that, nevertheless, a residual reducing ability derived from some source other than vitamin C may be present.

Crystallization of blood vitamin C as an osazone[4] is not practicable. To test the titration procedure a method to be described was therefore proposed. The basis of the method is the fact that many plant juices contain an enzyme which almost specifically catalyzes the oxidation of vitamin C. Press juice of cucumbers, for example, contains this enzyme in high concentration.[5-8]

The effectiveness of enzyme solutions obtained was tested on known ascorbic acid solutions under constant conditions. Boiled press juice was found in control tests to be without action. In preliminary experiments it could also be established that serum was without inhibitory action upon the enzyme. Experiments were then set up as follows: 1 ml. of cucumber juice was added to 3 ml. of serum. As a control, 1 ml. of water or boiled juice was added to 3 ml. of serum. The enzyme was allowed to act for fifteen minutes at room temperature.

TABLE I

ACTION OF CUCUMBER JUICE ON VITAMIN C IN SERUM AND IN PLASMA

NO.	VITAMIN C IN MG. PER CENT			
	SERUM + CUCUMBER JUICE	SERUM CONTROL	PLASMA + CUCUMBER JUICE	PLASMA CONTROL
1	Trace	0.9	Trace	1.1
2	0.12	0.7	Trace	1.3
3	0.14	1.1	Trace	1.2
4	0	0.6		
5	Trace	1.3		
6	Trace	1.3		
7	0	0.8		
8	0	1.4		
9	0	1.0		

Table I shows that the entire reducing ability of serum to Tillman's reagent is almost invariably abolished by the action of cucumber juice. The residual reducing power found in certain cases was almost completely accounted for by the reduction values found in the blank. It is right to conclude therefore that the method of Farmer and Abt gives a measure of the actual concentration of vitamin C in the serum and is not in practice subject to the interference of reducing agents, other than vitamin C.

A second aspect requiring investigation was the velocity of oxidation of vitamin C in the blood. The rapidity with which vitamin C in the urine and other biological fluids is decomposed and the difficulties caused thereby in the way of precise determination are known. We found that the speed of oxidation in serum is relatively much slower. The serum protein was easily shown to be responsible for the oxidation inhibition. Addition of serum to a pure ascorbic acid solution produced the full inhibitory effect; addition of deproteinized serum produced no inhibition whatsoever. The inhibitory effect observed is a com-

mon property of many colloids and is a result of their ability to bind copper by its conversion into an ineffective complex. Gelatin, agar, and gum arabic inhibit oxidation; pectin, on the other hand, stimulates oxidation.

TABLE II

INHIBITION OF OXIDATION BY ADDITION OF OXALATE

TIME HOURS	WATER	WATER + OXALATE ADDITION 2%	SERUM	PLASMA + OXALATE ADDITION
		Phosphate buffer pH 6.9		
		Ascorbic acid in mg. per cent		
0	1.60	1.65	0.82	0.82
1	1.25	1.64		
2	0.95	1.64		
3	0.62	1.62	0.43	0.47
24	0	1.46	Trace	Trace

Oxidation is inhibited but not suppressed by serum proteins. The loss in vitamin content of the serum may amount to 30 per cent or more within a period of four hours at room temperature. If it is impossible to carry out the analysis of blood samples immediately, the question whether oxidation of ascorbic acid in the blood can be suppressed by artificial additions therefore assumes practical interest. In controlled experiments it was shown that the addition of oxalate to pure ascorbic acid solutions almost completely inhibits the oxidation process (complex formation). This method would be simplest for blood. It was found, however, that the addition of oxalate to blood, i.e., to tested plasma samples, failed to inhibit oxidation.

TABLE III

EFFECT OF KCN (5 MG. PER CENT) ON THE OXIDATION OF VITAMIN C IN SERUM (PLASMA)

TIME HOURS	VITAMIN C IN MG. PER CENT							
	SERUM	SERUM + KCN	SERUM	SERUM + KCN	SERUM	SERUM + KCN	SERUM	SERUM + KCN
½	1.4	1.4					1.4	1.4
1	1.3	1.3	1.2	1.4	1.0	1.3		
2	1.1	1.2	1.1	1.3	0.7	0.7	1.20	1.23
3	1.0	1.0	1.0	1.0				
4							1.12	1.15
24			0.1	0.3			0.47	0.42
1	1.3	1.3						
2			0.7	0.8				
4			0.4	0.5				
8	0.3	Trace						

PURE ASCORBIC ACID SOLUTION, PHOSPHATE BUFFER PH 7.2

TIME HOURS	VITAMIN C IN MG. PER CENT	
	AQUEOUS SOLUTION	IN 1 MG. PER CENT KCN
0	2.26	2.26
½	1.04	2.24
2	0.51	2.24
24	0	2.00

In pure solutions and in many biological media, hydrocyanic acid, the best known retardant of heavy-metal catalyzed reactions, is generally used as an inhibitor of ascorbic acid oxidation. It seemed promising to test this method

in blood, the more so since this method has already been used for this purpose by several investigators.[9, 10] Careful analysis revealed, however, that suppression of oxidation of vitamin C in serum by addition of potassium cyanide is uncertain. Table III compares the effect of potassium cyanide in pure solutions with the effect in serum.

For several experiments, an inhibition of vitamin C oxidation in native undiluted serum by means of hydrocyanic acid could not be obtained. Recently, Farmer and Abt[11] reported their inability to obtain an inhibition of vitamin C oxidation in serum by means of potassium cyanide. The oxidation process in question which occurs equally well in the presence as in the absence of potassium cyanide is, therefore, not a process catalyzed by heavy metals, but an auto-oxidation.[12] A number of substances known for their ability to form complexes with heavy metals and which inhibit oxidation effectively in pure solutions were without influence on oxidation in serum. Heating to 60° C., it may be noted, accelerated the oxidation.

No practical and easy method of inhibiting the oxidation of vitamin C in stored serum was found. It is essential, therefore, to carry out determination of vitamin C in blood immediately after the sample is drawn. A delay of several hours in carrying out the titration may significantly reduce the vitamin concentration found. If the deproteinized blood cannot be analyzed immediately, the use of metaphosphoric acid as a deproteinization agent is advantageous. Table IV compares the values of vitamin C obtained after different times in blood which has been deproteinized by metaphosphoric acid and Folin-Wu reagent, respectively.

TABLE IV

TIME HOURS	VITAMIN C IN MG. PER CENT							
	HPO_3	FOLIN-WU	HPO_3	FOLIN-WU	HPO_3	FOLIN-WU	HPO_3	FOLIN-WU
1	1.3	1.1	1.4	1.4				
2	1.2	0.8	1.4	0.8	1.0	0.8	1.3	1.0
3			1.3	0.6				
4	1.1	0.41	1.4	0.4	0.9	0.6	1.0	0.4
24	0.8	0.1	0.9	0	0.7	0.2	0.9	0.1
2	1.4	1.1						
4	1.2	0.5						
24	1.1	0.2						

The experiments show that in brief intervals of storage metaphosphoric filtrates suffer only negligible losses; in Folin-Wu filtrates, on the other hand, oxidation is more rapid. No explanation for the superiority of metaphosphoric acid in this respect can be given.

Attempts were made in controlled experiments to influence the stability of vitamin C in serum by varying the H ion concentration. The effect of pH on the oxidation rate of vitamin C in pure solution, urine, tissue juice, etc., has long been known and used to advantage (e.g., determination in the presence of acetic acid). The pH relationship is, however, essentially different.

Controlled experiments showed that in 4 per cent serum solution the stability of vitamin C is maximal at neutral or weakly alkaline pH values. At increasing acidity, the inhibitory effect of the serum on the rate of oxidation becomes progressively weaker until at pH 5 it is at a minimum and practically

negligible. At this pH the stability of vitamin C in serum is least. Acidification of serum samples is, therefore, a disadvantage and not an advantage in analysis. Since the effect of KCN is much more marked in the acid pH range than in the alkaline range, it may be concluded that at acid pH heavy metal catalysis occurs.[13]

TABLE V

TIME HOURS	ASCORBIC ACID IN MG. PER CENT pH = 8 SERUM	pH = 8 CONTROL	pH = 7.2 SERUM	pH = 7.2 CONTROL	pH = 5.8 SERUM	pH = 5.8 CONTROL
0	2.30	2.30	2.35	2.00	2.40	2.10
1	2.11	1.45	2.22	0.98	1.55	1.16
2	2.10	1.16	2.12	0.62	1.15	0.74
3	2.00	0.57	1.90	0.23	0.72	0.20
24	1.15	0	1.05	0	0	0

TIME HOURS	pH = 8 SERUM	pH = 8 SERUM + KCN	pH = 8 BLANK	pH = 5 SERUM	pH = 5 SERUM + KCN	pH = 5 BLANK
0	1.80	1.85	1.46	1.78	1.76	1.48
2	1.75	1.83	0.45	0.35	1.34	0.30
3	1.65	1.78	0.13	0	1.25	0
24	0.59	0.66	0	0	0.31	0

SUMMARY

It has been shown that the behavior of vitamin C in serum is distinctive. The oxidation of the vitamin is inhibited markedly by the serum proteins.

The stability of the vitamin (i.e., the inhibition of oxidation) is greatest at neutral or weakly alkaline pH. On the acid side, the inhibitory effect diminishes until at pH 5 it is minimal (controlled experiments in 4 per cent serum).

Potassium cyanide and other substances of similar activity do not inhibit the residual auto-oxidation of vitamin C in serum. No method of inhibiting this residual oxidation could be found. The determination of vitamin C in the blood must, therefore, be carried out immediately after the blood is drawn. Within a few hours, losses have already occurred. In this respect, deproteinization by metaphosphoric acid presents an advantage, since vitamin C in metaphosphoric acid filtrates remains intact for several hours, whereas in Folin-Wu filtrates under similar conditions it is unstable.

By the action of ascorbic acid oxidase, the reducing power of serum toward Tillman's reagent is practically reduced to zero. The reducing power of serum toward this reagent, therefore, constitutes a valid measure of its vitamin C content. The action of oxidase is not influenced by serum but heavy metal catalysis is inhibited by serum.

II. CLINICAL PART

During the period between September 8, 1936 to September 24, 1937, the blood vitamin C content of 322 women was examined. The average concentration of vitamin C was found to be 1.01 mg. per cent.

Table VI shows that the average vitamin C concentration of the blood is highest during pregnancy, and lowest after confinement. The latter values, however, were all obtained during August and September, and since at this time of the year the blood vitamin concentration in women is not yet at its normal level, conclusions based on the figures obtained are necessarily tentative.

TABLE VI

CONCENTRATION OF VITAMIN C IN BLOOD OF HUMAN FEMALES

DESCRIPTION OF SUBJECT	NUMBER OF CASES	AVERAGE BLOOD VITAMIN C CONCENTRATION IN MG. PER CENT
Nonpregnant	47	0.98
Pregnant	207	1.09
After confinement	62	0.787
Total	322	1.01

In Table VII the vitamin level at different months of the year is indicated. The highest values were obtained between October and February inclusive, i.e., during the citrus season; after this the values gradually decreased. This finding, therefore, constitutes further proof of the close correlation between the diet and the concentration of vitamin C in the blood.

TABLE VII

CONCENTRATION OF VITAMIN C IN BLOOD DURING DIFFERENT MONTHS OF THE YEAR IN PALESTINE

MONTH		NO. CASES TESTED	AVERAGE BLOOD VITAMIN C CONCENTRATION IN MG. PER CENT
October	1936	16	1.24
November	1936	38	1.12
December	1936	19	1.27
January	1937	16	1.29
February	1937	28	1.21
March	1937	9	1.16
April	1937	16	1.09
May	1937	36	0.95
June	1937	32	1.04
July	1937	47	0.95

Among the 207 pregnant women examined, 7 serious cases of hyperemesis gravidarum were found. Whereas the average vitamin concentration in the blood of pregnant women was found in a total of 207 cases to be 1.09 mg. per cent, the average concentration of vitamin C in the blood of the 7 women suffering from vomitus gravidarum was 0.70 mg. per cent; the deviations from this figure were very small.

TABLE VIII

CONCENTRATION OF VITAMIN C IN THE BLOOD OF WOMEN DURING HYPEREMESIS GRAVIDARUM

NUMBER OF CASES TESTED	DESCRIPTION	AVERAGE CONCENTRATION OF VITAMIN C IN BLOOD IN MG. PER CENT
207	Pregnant	1.09
7	Cases of hyperemesis gravidarum	0.70

It may be suggested on the basis of experience, that the low concentration of vitamin in these cases is a secondary rather than a primary symptom, and is the result of a vitamin shortage created in the blood by continuous vomiting and deficient nutrition.

Recently the treatment of hyperemesis gravidarum by vitamin C has been suggested. Schmidt,[14] Dicker,[15] and others reported success in the use of large vitamin C doses.

Of the 7 patients we encountered, 4 were treated with vitamin C, only one of whom was successful.

Four of the patients were beneficially affected simply by the different environment provided by hospital life, and ceased to vomit four days after admission to the hospital, even without special treatment. One of these 4 had been treated at her home with large doses of vitamin C but with little success. All 4 showed low blood vitamin concentration, the rates being 0.61, 0.65, 0.70, and 0.88 mg. per cent, respectively.

The data collected seem to justify the following conclusions: (1) Administration of vitamin C is not a specific treatment for hyperemesis gravidarum. (2) The low concentration of vitamin C in the blood is a secondary symptom evoked by undernutrition.[16]

In 80 out of 207 pregnant women observed, the state of health of the teeth and the calcium and phosphorus concentration of the blood were determined.

Stepp, Kühnau, and Schroeder[17] expressed the opinion that vitamin C deficiency is primarily responsible for structural teeth deficiencies.

Gaethgens[18] regarded dental caries a symptom of vitamin C deficiency during pregnancy. The severity of the caries in pregnancy, according to this author, is also influenced by vitamin D and calcium deficiency.

Weibel[19] and Seitz[20] both attributed caries during pregnancy to calcium deficiency.

McIlroy[21] and the American Research Committee[22] arrived at the conclusion that "mineral starvation" is responsible for caries. These authors rejected the possibility that vitamin C deficiency is the cause, since vitamin C failed in their experiments to influence the condition in pregnancy.

Data on the state of health of the teeth in pregnancy are classified in Table IX as good, medium, or bad (cases of caries or pulpitis teeth).[27]

TABLE IX

STATE OF HEALTH OF TEETH	NUMBER OF PATIENTS	VITAMIN C AVG.	P AVG.	CA AVG.	AGE AVG.
Good	17	1.13	3.88	10.39	22.2
Medium	27	1.00	3.65	10.39	21
Bad	36	1.01	3.82	10.51	28.8
Total	80	1.05	3.56	10.45	

The concentration of vitamin C in the blood of women is seen to be independent of the state of teeth. There was likewise no difference with respect to the concentration of phosphorus or calcium in the blood. Consistently higher concentrations of vitamin C were recorded in the blood of pregnant women with healthy teeth, but the increase was so small that no certain conclusions can be based on these findings.

The question whether there is a relationship between gingivitis and vitamin C was of some interest. In the recent literature Stepp, Kühnau, and Schroeder[17] state that the cause of bleeding of the gums is vitamin C deficiency.

Seitz[20] found that in about one-half the pregnant patients he examined, a marked swelling and reddening of the gums and a tendency to bleeding set in after the fifth month.

Gaethgens[18] assumed that vitamin C deficiency during pregnancy manifests itself as latent scurvy. Bleeding of the gums is supposed to be the only symptom of the latent disease.

Of the 207 pregnant patients we examined, 43 cases of bleeding gums in women of different ages were recorded. The concentration of vitamin C in the blood of these patients was in no case far from the average of 1.18 mg. per cent. In 7 of the 43 patients, gingivitis was found to be associated with caries. The values obtained in such cases for blood vitamin C were: 0.67, 0.69, 0.95, 0.97, 1.00, 1.00, and 1.05 mg. per cent, respectively. The average value was 0.90 mg. per cent, and thus somewhat below normal. The number of observations is too small, however, to permit final conclusions.

The foregoing results lead to the conclusion that vitamin C deficiency is not the cause of gingivitis during pregnancy. The values of vitamin C in gingivitis are definitely not below normal.

Attention was directed to the concentration of vitamin C in the milk of human mothers after confinement. Sixty-two women were examined during the period from August to September, 1937. The examination was conducted among the patients of the Rothschild Hadassah Hospital, all of whom received the same nourishment. The average concentration of vitamin C in the milk was found to be 4.62 mg. per cent, a value corresponding closely to that indicated in the literature (Bleyer, Cahnmann, and Schlemmer,[23] Harris and Ray,[24] Wachholder,[25] Ferdinand[26]).

One hundred forty-seven tests of the milk of 62 mothers on the first nine days after confinement were carried out. The results are summarized in Table X. A continuous increase in the vitamin C concentration of the milk from the first day on was noted. The quantity of vitamin C obtained by the infants on successive days after birth could be calculated.

TABLE X

Days after birth	1	2	3	4	5	6	7	8
Concentration of vitamin C in the milk, average value in mg. per cent	2.63	3.48	5.41	6.27	5.55	5.86	5.48	5.52
Quantity of milk in gm.		70	140	210	280	350	420	490
Quantity of vitamin C obtained in milk in mg.		2.436	7.57	13.16	15.54	20.51	23.01	27.0

The question whether a relationship exists between the concentration of vitamin C in the blood and in the milk seemed to be of some importance. To decide this point, the data on the concentration of vitamin C in the blood were divided into two categories: patients with vitamin values below 0.75 mg. per cent, and patients with vitamin values above 0.75 mg. per cent. The results are recorded in Table XI.

TABLE XI

NO. OF CASES	CONCENTRATION OF VITAMIN C IN BLOOD MG. PER CENT	CONCENTRATION OF VITAMIN C IN MILK MG. PER CENT
66	Below 0.75	5.1
77	Above 0.75	5.5

Table XI shows that the concentrations of vitamin C in milk and in blood are not closely correlated. The impression was obtained that the vitamin concentration in the milk is maintained at the normal level even when the concentration in blood is below normal. This unexpected possibility will be tested more closely in experiments to be undertaken. According to statements in the literature, a correlation exists between the concentration of vitamin in the milk and in the urine as measured in the repletion test.

It was established that the age of the mother, the number of children previously born, and the weight of the born child do not influence the concentration of vitamin C in blood or in milk.

SUMMARY

The aim of this paper has been to show the importance of the estimation of vitamin C level in the blood for the exact diagnosis of a real vitamin C deficiency.

The concentration of vitamin C in the blood of 322 women (pregnant, post partum, nonpregnant) was measured. The average concentration was found to be 1.01 mg. per cent.

In pregnant women (207 cases) the average blood concentration of vitamin C was 1.09 mg. per cent; in post-partum women (62 cases), the average concentration was 0.79 mg. per cent; in nonpregnant women (47 cases), the average concentration was 0.98 mg. per cent.

In severe cases of hyperemesis gravidarum, the blood concentration of vitamin C was consistently low and averaged 0.7 mg. per cent. The low concentration is believed to be of secondary origin. Administration of vitamin C is therefore not causal therapy.

The concentration of vitamin C in the blood of pregnant women was found to be 1.13 mg. per cent when the teeth were in good condition (17 cases); 1.00 mg. per cent when the teeth were in medium condition (27 cases); and 1.01 mg. per cent when the teeth were in bad condition (36 cases).

The concentration of vitamin C in the blood of pregnant women suffering from gingivitis (43 cases) was normal, rating 1.18 mg per cent.

The blood concentration of vitamin C when gingivitis was complicated by caries (7 cases) was slightly below normal, rating 0.9 mg. per cent.

The concentration of calcium and phosphorus in the blood of pregnant women was found to be independent of the condition of the teeth.

The concentration of vitamin C in human milk within the first nine days after confinement was measured (62 cases and 147 tests). The average concentration was found to be 4.62 mg. per cent.

A continuous increase in the quantity of vitamin C in the milk beginning from the first day after birth was observed.

The quantity of vitamin C excreted in the milk per day was calculated to be 2.4 mg. on the second day, and 27 mg. on the eighth day post partum.

The vitamin C concentrations of milk and blood were not found to be definitely correlated.

The age of the mother, the number of children previously borne, and the weight of the newborn infant do not influence the concentration of vitamin C in the milk and in the blood.

The blood concentration of vitamin C after confinement was below normal (less than 0.75 mg. per cent) in 50 per cent of the patients examined. In certain patients, values as low as 0.32 mg. per cent were encountered.

A survey of the concentration of vitamin C in the blood during different months of the year revealed the existence of a markedly higher average during the citrus season.

REFERENCES

1. Zilva, S. S.: Vitamin C Requirements of Guinea-Pig, Biochem. J. **30**: 1419, 1936.
2. Faulkner, I. M., and Taylor, F. H. L.: Observations on Renal Threshold for Ascorbic Acid in Man, J. Clin. Investigation **17**: 69, 1938.
3. Farmer, C. I., and Abt, A. F.: Ascorbic Acid Content of Blood, Proc. Soc. Exper. Biol. & Med. **32**: 1625, 1935.
4. Hinsberg, K.: Die Stabilisierung der Ascorbinsäure durch Metaphosphorsäure, Biochem. Ztschr. **290**: 125, 1937.
5. Tauber, H., Kleiner, I. S., and Mishkind, D.: Ascorbic Acid (Vitamin C) Oxidase, J. Biol. Chem. **110**: 211, 1935.
6. Szent-Györgyi, Albert: On the Function of Hexuronic Acid in the Respiration of the Cabbage Leaf, J. Biol. Chem. **90**: 385, 1931.
7. Johnson, S. W., and Zilva, S. S.: Relation Between Rate of Enzymic Oxidation and Stereochemical Structure of Ascorbic Acid and Its Analogues, Biochem. J. **31**: 1366, 1937.
8. Srinivasan, M.: Enzymic Determination of Ascorbic Acid, Biochem. J. **31**: 1524, 1937.
9. Pijoan, M., and Klemperer, F.: Determination of Blood Ascorbic Acid, J. Clin. Investigation **16**: 443, 1937.
10. Pijoan, M., and Eddy, E.: Ascorbic Acid Content of Red Cells and Plasma, J. Lab. & Clin. Med. **22**: 1227, 1937.
11. Farmer, C. I., and Abt, A. F.: Invalidation of Plasma Ascorbic Acid Values by Use of Potassium Cyanide, Proc. Soc. Exper. Biol. & Med. **38**: 399, 1938.
12. Barron, E. S. G., De Meio, R. H., and Klemperer, F.: Studies on Biological Oxidations; Copper and Hemochromogens as Catalysts for Oxidation of Ascorbic Acid. Mechanism of Oxidation, J. Biol. Chem. **112**: 625, 1936.
    Barron, E. S. G., Barron, A. G., and Klemperer, F.: Studies on Biological Oxidations; Oxidation of Ascorbic Acid in Biological Fluids, J. Biol. Chem. **116**: 563, 1936.
13. Boyden, R., and Potter, V. R.: On Form of Copper in Blood Plasma, J. Biol. Chem. **122**: 285, 1938.
14. Schmidt, Walter (Halle/Saale): Neue Wege in der Behandlung des Schwangerschaftserbrechens, München. med. Wchnschr. **83**: 357, 1936.
15. Dicker, S.: Le traitement des vomissements de la grossesse par l'acide l-ascorbique (vitamine C). Le facteur thyréo-surrénalien dans les phénomènes d'intolérance, Schweiz. med. Wchnschr. **67**: 74, 1937.
16. Sadowsky, A.: Vitamin C Level in Blood in Cases of Persistent Vomiting During Pregnancy and Treatment of Such Cases With Vitamin C, Harefuah **14**: x, 1938.
17. Stepp, W., Kühnau, J., and Schroeder, H.: Die Vitamine und ihre klinische Anwendung, Stuttgart, 1936, Ferdinand Enke.
18. Gaethgens, G.: Der Vitaminhaushalt in der Schwangerschaft, Leipzig, 1937, Theodor Steinkopff.
19. Weibel, W.: Lehrbuch der Frauenheilkunde, Band I: Geburtshilfe, Berlin, 1937, Urban & Schwarzenberg.
20. Stoeckel, W.: Lehrbuch der Geburtshilfe, ed. 4, Jena, 1935, Gustav Fischer.
21. McIlroy, L.: The Toxemias of Pregnancy, London, 1936, Edward Arnold & Co.

22. Hollander, Franklin: Coordinated Report of the Columbia University Dental Caries Research Group, February, 1930—February, 1934, J. Dent. Research **14**: 303, 1934.
23. Schlemmer, F., Bleyer, B., and Cahnmann, H.: Studien über biochemische Aktivatoren der Milch: die Beziehungen zwischen dem Reduktionsvermögen der Milch und ihrem Gehalt an Vitamin C; die Auswertung der Milch auf Vitamin C nach chemischer Methode, Biochem. Ztschr. **254**: 187, 1932.
24. Harris, L. J., and Ray, S. N.: Diagnosis of Vitamin C Subnutrition by Urine Analysis, With Note on Antiscorbutic Value of Human Milk, Lancet **1**: 71, 1935.
25. Wachholder, K.: Die Versorgung des Säuglings mit Vitamin C, Klin. Wchnschr. **15**: 593, 1936.
26. Ferdinand, H.: Der Vitamin C-Gehalt der Frauenmilch und der Kuhmilch in den Frühjahrsmonaten, Klin. Wchnschr. **15**: 1311, 1936.
27. Sadowsky, A., and Wallach, J.: The Relation Between Vitamin C, Phosphorus and Calcium Content of Blood and Condition of the Teeth in Pregnant Women, Harefuah **15**: x, 1938.

MAGNESIUM: THE EFFECTS OF INTRAVENOUS INJECTIONS ON THE HUMAN HEART*

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VERY little work is available concerning the parenteral effects of magnesium on the heart. However, it is not uncommon to run across such statements in the literature that "sudden death following the injection of a magnesium salt . . . is not an uncommon occurrence."[1] The literature has recently been summed up by Miller and Van Dellen, who in animal experiments found deleterious effects by magnesium on the cardiac conduction system.[1] The doses they used, however, were massive. In view of the increasing use of magnesium in the treatment of various types of cardiac disease,[2] and even more so in view of the great importance magnesium has assumed recently as a circulation time agent,[3, 4] we thought it apropos to determine in a systematic study the intravenous effects of *therapeutic* doses of magnesium on the human heart.

METHOD

One hundred adult patients were used in this investigation. One group of 34 cases was apparently free of cardiovascular disease; another group of 66 cases presented various forms and degrees of cardiac disease as outlined below. A total of 104 sets of electrocardiographic tracings were taken—69 in the cardiac group and 35 in the noncardiac group.

The three standard leads of the electrocardiogram were taken first. The electrocardiographic circuit was completed again, and simultaneously with the taking of Lead I, 10 c.c. of a warmed 10 per cent aqueous solution of magnesium sulfate were injected into a large antecubital vein of the reclining patient. In accordance with the technique of the magnesium circulation-time test,[4] the first 6 c.c. were injected through a No. 18 gauge needle as rapidly as possible (requir-

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TABLE I

ILLUSTRATIVE ELECTROCARDIOGRAPHIC EFFECTS OF INTRAVENOUS MAGNESIUM SULFATE INJECTIONS

CASE	NAME	SEX	AGE	BLOOD PRESSURE		TRACING I DEPARTURES FROM NORMAL	TRACING II CHANGES DURING MAGNESIUM INJECTION	TRACING III CHANGES ONE HOUR AFTER INJECTION	REMARKS
				SYST.	DIAST.				
112	A. L.	M	50	104	60	None	T_3 raised (was flat)	QRS_1 decreased amplitude; T_2 raised (was flat); QRS_3 increased amplitude	Noncardiac patient Duodenal ulcer
56	A. F.	F	61	160	78	P waves low; QRS slightly slurred W QRS_3	QRS_2 moderately increased amplitude; W QRS_3 more marked	None	Arteriosclerotic heart disease
70	M. G.	M	63	126	40	Right axis deviation; QRS slurred and widened to 0.12 second; S_1 pronounced in all tracings; T_1 inverted	Absence of S_2; R_2 amplitude markedly increased	Absence of S_2; R_2 amplitude markedly increased	Acute coronary thrombosis; heart block (2:1 to 3:1) delayed conduction; arteriosclerotic heart disease
172	M. S.	M	67	176	78	QRS slurred; QRS_2 W shaped and negative; $T_{2\text{-}3}$ inverted; T_1 flat	T_2 flat (higher); QRS_2 became positive and lost the W type	T_2 flat (higher); QRS_2 became positive and lost W	Hypertensive heart disease; angina pectoris
53	B. E.	F	50	230	120	Left axis deviation; QRS slightly slurred; Q_3 deep; T_3 inverted	QRS M shaped and more slurred	None	Hypertensive heart disease
8	M. A.	F	54	240	140	QRS slurred; $T_{1,2}$ diphasic; left axis deviation	None	QRS_2 markedly decreased amplitude; QRS_3 increased amplitude	Old posterior coronary thrombosis; hypertensive heart disease
221	R. N.	M	19	94	60	Complete heart block (11/9/37); auricular rate 83, ventricular rate 30; QRS prolonged to 0.18 and slurred; T_1 inverted	None	QRS_3 narrower and higher (deeper)	Postscarlatinal complete heart block; three previous sets of tracings showed no changes

Tracing I—Set of three standard leads taken before injection of magnesium sulfate
Tracing II—Set of three standard leads taken during injection of magnesium sulfate
Tracing III—Set of three standard leads taken one hour after injection of magnesium sulfate

ing between one and one-half and two seconds), and the balance was injected more slowly. In a few cases 20 c.c. instead of 10 c.c. were injected. No injection required more than ten seconds, and most injections required considerably less. In succession, Leads II and III were then recorded, the total time elapsing from the moment of injection until the completion of Lead III being approximately three minutes. The time was spaced equally between the three leads. One hour later the standard leads were again recorded. Three sets of tracings were thus available for each case: a control set, a set taken during and closely after the injection, and a set one hour after the injection. These three sets are hereafter referred to as tracing I, tracing II, and tracing III, respectively. Space does not permit the publication of all of the available data, but illustrative cases are herein presented.

Noncardiac Group.—The group of noncardiac patients comprised 34 cases suffering from a wide assortment of diseases. It included 18 females and 16 males, ranging in age from 19 to 73 years. All were free of cardiovascular disease as ascertained by history, physical examination, and electrocardiographic evidence. Thirty-five sets of tracings were taken.

Ten patients showed electrocardiographic changes. Four patients showed delayed effects (that is, in tracing III); six patients developed changes both during the injection period and one hour later. No typical electrocardiographic pattern was observed. During the injection period Lead I was affected in only one case (Case 209) which showed a slight decrease in amplitude T_1. In one case (Case 61) T_3 was slightly decreased in amplitude; the reverse held true in another case (Case 112). The other changes consisted in very slight variations in amplitude of QRS_2 and QRS_3. The development in one case (Case 79) during the injection period of occasional U waves in Lead II, which subsequently disappeared, was interesting.

At no time were changes noted in the P waves, the duration of the P-R interval, or the heart rate. Case 112, Table I, had two sets of tracings, of which one showed no changes, and the other minor T and QRS changes. All other cases showed usually one minor variation.

Cardiac Group.—The cardiac group comprised 66 patients, 35 females and 31 males, ranging in age from 16 to 75. Sixty-nine sets of tracings were taken. One patient (Case 221, Table I) had four sets of tracings.

Arteriosclerotic Heart Disease.—Twenty-eight patients fell in the arteriosclerotic heart disease group; 9 had coexistent diabetes mellitus, of whom 2 had developed acute anterior coronary thrombosis; 3 had healed posterior coronary thrombosis; 2 had partial heart block (one complicated by an acute coronary thrombosis); one had an acute posterior coronary thrombosis; and 3 had auricular fibrillation. Two cases were complicated by pulmonary emphysema (one with bronchial asthma).

Of 10 patients in the arteriosclerotic heart disease group presenting electrocardiographic changes, only 2 showed changes during the injection period alone; 2 showed changes both during the injection and one hour later, and the balance showed deferred changes only. During the injection period the changes

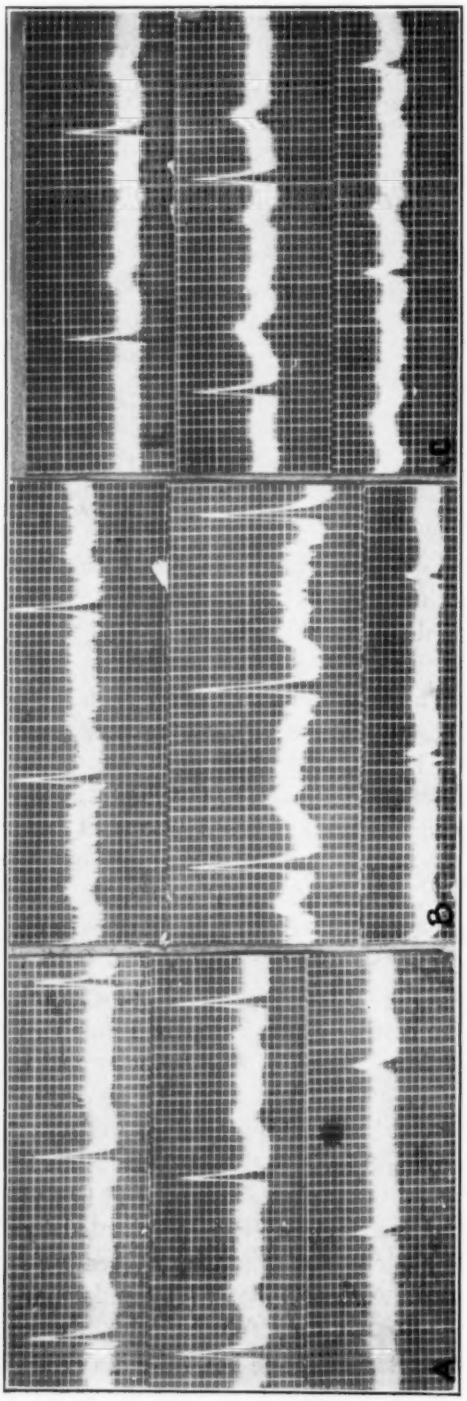

Fig. 1.—Case 56. *A*, Control before magnesium injection. *B*, Increase in QRS amplitude in Lead II and W QRS Lead III during magnesium injection. *C*, No change one hour after magnesium injection.

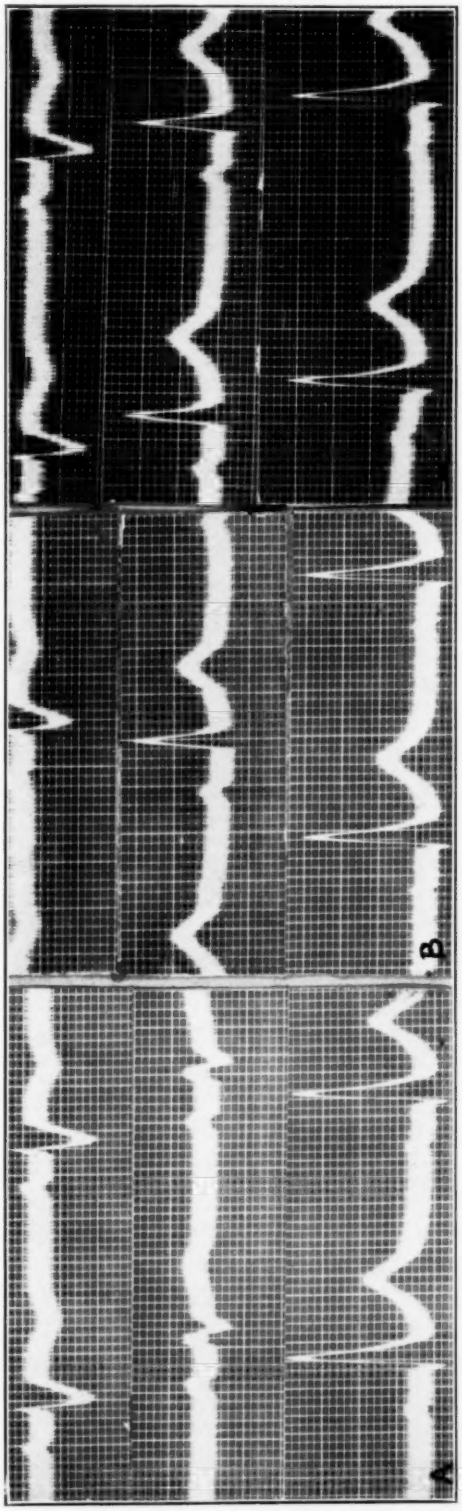

Fig. 2.—Case 70. *A*, Control before magnesium injection. *B*, Increased R_2 and absent S_2 during magnesium injection. *C*, Same as *B* one hour after magnesium injection.

were limited to increase of QRS_2 amplitude and accentuation of W QRS_3 (Case 56, Fig. 1), and increase in amplitude of T_3 (Case 5). Case 70, shown in Fig. 2, presented a marked increase in R_2 amplitude as well as an absence of S_2 in both tracings II and III. One case (Case 3) developed a slight increase in T_2 amplitude in tracings II and III. In the deferred change group (tracing III) were decrease of QRS_3 amplitude (Case 49) and an interesting case (Case 168) in which QRS_3 changed from slurred to W. In one case (Case 13) QRS became lowered in amplitude and W shaped; in another case (Case 64) QRS_2 became lower in amplitude with marked slurring. The left axis deviation present in one case became more marked (Case 72). T_2 became inverted in one case (Case 102).

Hypertensive Cardiovascular Heart Disease.—The hypertensive cardiovascular disease group comprised 25 patients; 3 had coexistent diabetes mellitus (one with partial heart block), one had central nervous system syphilis, one hyperthyroidism, one angina pectoris, one had chronic glomerulonephritis, one auricular fibrillation, one a recent anterior coronary thrombosis, one an old posterior coronary thrombosis, and one an acute anterior coronary thrombosis.

Eleven patients in the hypertensive group presented electrocardiographic changes. Two developed changes during the injection period, 6 showed changes as deferred effects and 3 showed changes during the injection period and one hour later. During the injection period one patient (Case 35) developed an increase in amplitude of QRS_1; in another case (Case 53, Fig. 3) the QRS_2 complexes became M shaped with increased slurring. One case (No. 31) in which QRS_3 was inverted and W shaped became positive in tracing II and showed a mixture of positive and negative complexes in tracing III. Case 65 showed an increase in QRS_2 amplitude with increased slurring in tracing II, an effect which disappeared in tracing III, only to be replaced by deepening of the T_1 and T_2 waves. Another interesting case (Case 172, Fig. 4) displayed an increase of T_2 amplitude in tracings II and III as well as QRS_2 becoming positive and losing the W. As deferred effects (tracing III) were: QRS showed slightly decreased amplitude in one case (Case 37); QRS_2 decreased and QRS_3 increased in amplitude in one case (Case 8, Fig. 5); QRS_1 amplitude increased and the QRS_3 complex became inverted in another case (Case 69); QRS_3 changed from negative to positive (Case 171); QRS_1 showed slightly increased slurring in one case (Case 212), and the T waves increased slightly in voltage in another case (Case 84).

Miscellaneous Cases of Cardiac Disease.—Of a miscellaneous group comprising 8 patients there were 2 with chronic rheumatic heart disease, one with a postscarlatinal complete heart block, one with a syphilitic dissecting aneurysm of the arch of the aorta, 2 with congenital heart disease (the heart of one was complicated by a subacute bacterial pulmonary arteritis), one with hyperthyroidism, and one with a subacute bacterial endocarditis; two patients showed electrocardiographic changes during the injection period and one patient showed changes as a delayed effect. One of the foregoing cases (Case 188) developed inversion of T_1 and T_2, another (Case 145) an increase of R_3 amplitude during the injection period, and a third (Case 60) a marked decrease of R_2 amplitude as a de-

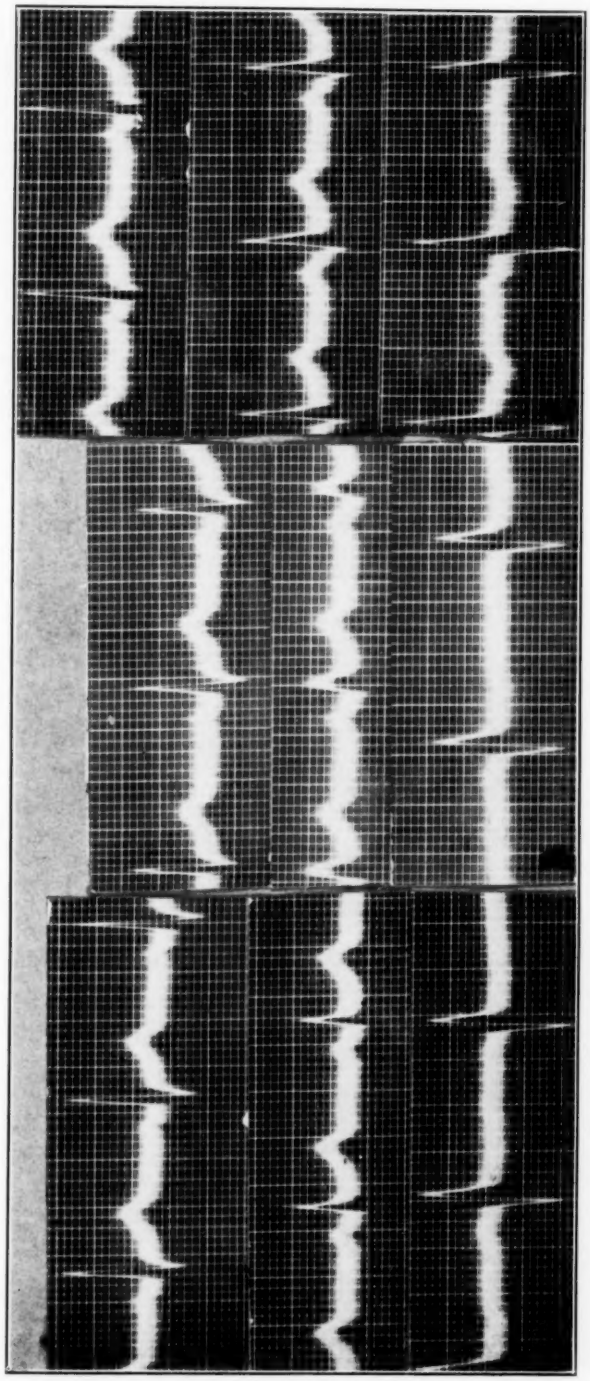

Fig. 3.—Case 53. *A*, Control before magnesium injection. *B*, QRS_2 has become more slurred and M shaped during magnesium injection. *C*, Showing reversion to control one hour after magnesium injection.

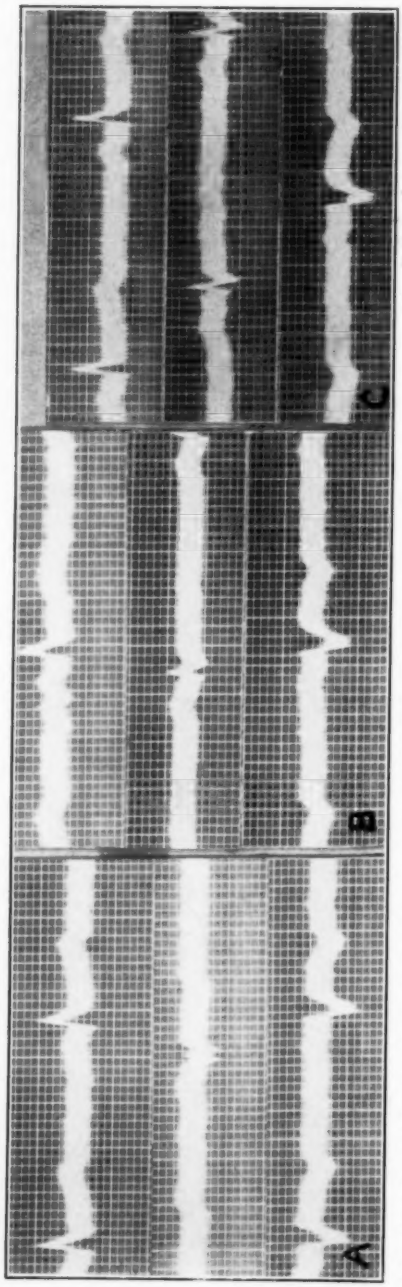

Fig. 4.—Case 172. *A*, Control before magnesium injection. *B*, QRS_2 became positive and lost W type. T_2 changed from inversion to flat during magnesium injection. *C*, Same changes as noted in *B* one hour after injection.

layed effect. A case of complete heart block (Case 221)[9] had four sets of tracings, of which the first three showed no changes, and the fourth a narrowing and deepening of the QRS_3 complex one hour after the injection.

Equivocal Heart Disease.—In a group of 5 young adults with electrocardiographic changes as the only evidence of cardiac disease, no changes developed during the injection period. One patient (Case 126) developed a sinus arrhythmia and one patient (Case 113) a slightly deeper Q_3 wave in tracing III.

In résumé, the cardiac group as a whole showed slight electrocardiographic changes in 26 of the 66 cases. Only 6 patients were limited to the injection period, 5 to effects both during the injection and subsequently, and 15 patients to the deferred period only. An outstanding feature is that the changes were confined almost exclusively to the T waves and QRS complexes. Even more noteworthy is the fact that during the true injection period (shown only in Lead I of tracing II), only two minor changes were recorded in the entire series. There were no P wave changes, no changes in the P-R interval or in the heart rate. No ventricular premature beats were noted. There were no bradycardias or cases of sinus arrest. Eleven patients who were receiving digitalis at the time of the injections, showed no untoward effects.

COMMENT

The striking feature is the complete lack of uniformity of changes wrought upon the heart by magnesium. That magnesium may have a direct effect upon the myocardium of the ventricles is indicated by the slight changes in QRS complexes and T waves occurring in a fairly high percentage of cases. The types of changes produced during the injection period and one hour later are practically the same, whether in the cardiac or noncardiac groups. That these changes are negligible is shown by the fact that Lead I (taken during the injection when the full brunt of the magnesium was borne) showed only one slight T_1 change in the noncardiac group and only one slight QRS_1 and one T_1 change in 2 of the cardiac cases. Moreover, very few patients showed more than one lead change whether during the injection or one hour afterwards. In 2 patients, one a noncardiac person and the other a critically ill complete heart block individual, in whom two sets and four sets of tracings were obtained respectively, it is noteworthy that minor changes were obtained in only one set of tracings in each patient. These results illustrate the inconstancy of magnesium effect. In general, there were as many changes in T waves and QRS complexes in one direction as there were in the opposite. Finally, such changes as did occur were evanescent inasmuch as standard electrocardiograms, taken in the vast majority of the patients within a few days after the injection, showed all magnesium effects to have vanished entirely. Consequently, we must conclude that the rapid intravenous injection of 10 per cent magnesium sulfate exerts no deleterious action on the heart.

It is interesting to compare these results with the cardiac effects induced by the intravenous use of 20 per cent calcium gluconate, a substance that has come into wide use recently as a circulation-time reagent. In normal individuals

calcium produces flattening or inversion of the T waves in 92 per cent of cases, flattening or inversion of the P waves in 54 per cent, and a marked bradycardia in 67 per cent.[5] No systematic studies of the cardiac effects of intravenous calcium injections in cardiac patients are available, so that we have no basis for direct comparison of the effects of magnesium and calcium in cardiac disease. That calcium injections clinically may lead to catastrophes in both normal and diseased hearts is now well substantiated.[5-8]

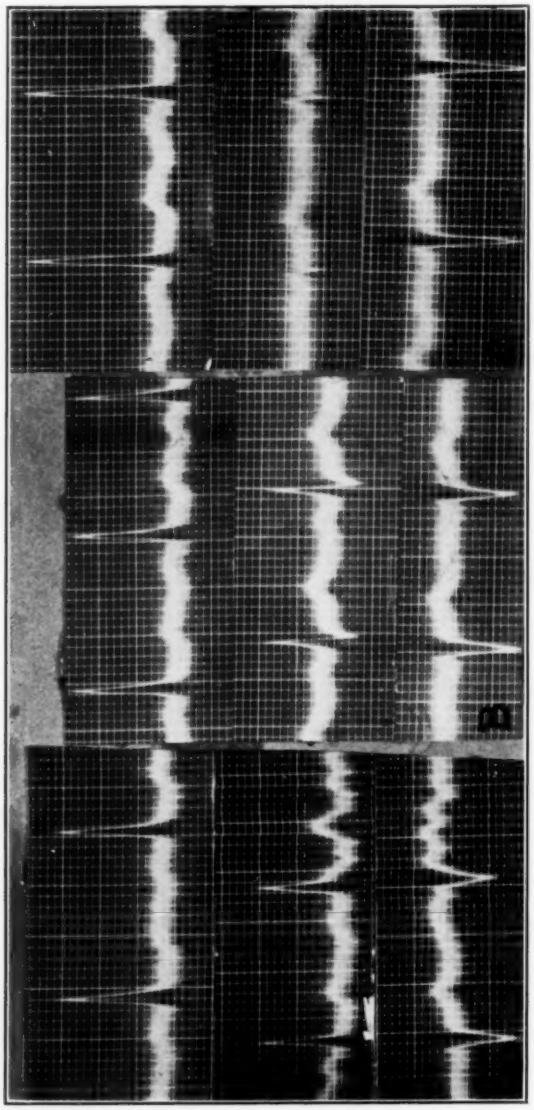

Fig. 5.—Case 8. *A*, Control before magnesium injection. *B*, QRS_2 lower voltage and QRS_3 increased voltage during magnesium injection. *C*, QRS_2 depressed and QRS_3 increased one hour after magnesium injection.

SUMMARY

1. The effects of the rapid intravenous injection of 10 c.c. of 10 per cent magnesium sulfate on the human heart were recorded electrocardiographically in 100 patients. The three standard leads of the electrocardiogram were re-

corded prior to injection, simultaneously with the injection, and one hour later. Thirty-five sets of tracings were taken in 34 noncardiac patients and 69 sets in 66 cardiac patients.

2. The cardiac group included 28 persons with arteriosclerotic heart disease, 25 persons with hypertensive heart disease, 5 persons with only electrocardiographic evidence of cardiac disease, and a miscellaneous group of 8 cases comprising 2 persons with chronic rheumatic heart disease, one with postscarlatinal complete heart block, one with syphilitic dissecting aneurysm of the aortic arch, two with congenital heart disease (one complicated by a subacute bacterial pulmonary arteritis), one with subacute bacterial endocarditis, and one with hyperthyroidism.

3. In the noncardiac group 6 patients presented minor T and QRS changes (usually in one lead) both during the injection period and subsequently; 4 persons showed similar changes one hour after injection.

4. In the cardiac group similar changes were exhibited by 6 persons during the injection period, by 15 persons one hour after injection, and by 5 persons both during the injection period and one hour later.

5. A discussion of the changes produced leads to the conclusion that intravenous magnesium injections exert no deleterious effect on the human heart. The coincidental administration of digitalis yields no untoward effects.

REFERENCES

1. Miller, J. R., and Van Dellen, T. R.: Electrocardiographic Changes Following the Intravenous Administration of Magnesium Sulfate. Experimental Study on Dogs, J. Lab. & Clin. Med. **23**: 914, 1938.
2. Zwillinger, L.: Über die Magnesiumwirkung auf das Herz, Klin. Wehnschr. **14**: 1429, 1935.
3. Neurath, O.: Untersuchungen uber die Bestimmung der Blumtumlaufsge-schwindigkeit mit Magnesiumsulfat, Ztschr. f. klin. med. **132**: 134, 1937.
4. Bernstein, Mitchell, and Simkins, Samuel: The Use of Magnesium Sulfate in the Measurement of Circulation Time, Am. Heart J. **17**: 218, 1939.
5. Berliner, Kurt: The Effect of Calcium Injections on the Human Heart, Am. J. M. Sc. **191**: 117, 1936.
6. Berliner, Kurt: Effect of Calcium on Heart, Am. Heart J. **8**: 548, 1933.
7. Hoff, A. E., and Nahun, L. H.: An Analysis of the Cardiac Irregularities Produced by Calcium and Their Preventions by Sodium Amytal, J. Pharmacol. & Exper. Therap. **60**: 425, 1937.
8. Golden, J. S., and Brams, W. A.: Mechanism of Toxic Effects From Combined Use of Calcium and Digitalis, Ann. Int. Med. **11**: 1084, 1938.
9. Bernstein, Mitchell: Auriculoventricular Dissociation Following Scarlet Fever, Am. Heart J. **16**: 582, 1938.

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CHOLESTEROLYSIS IN THE BLOOD PLASMA OF INDIVIDUALS WITH MENTAL DISORDERS*

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IN A SERIES of papers it has been demonstrated by one of us (Schube[1-3]) that in abnormal mental states as a group and in its various subdivisions there are certain alterations in cholesterol metabolism which are reflected in the whole blood cholesterol. In additional papers[4, 5] there were also reported studies on cholesterolysis in normal man. In them certain facts were brought out relative to the stability of cholesterol in blood plasma. In view of the cholesterol values in abnormal mental states and of our observations on cholesterolysis in normal man, we feel it pertinent to present a study on cholesterolysis in the blood plasma of individuals with abnormal mental states in the hope of further clarifying the problem of cholesterol metabolism.

METHOD

The individuals used for this work were all normal physically, but sufficiently mentally ill to necessitate their institutionalization. All were males, females being excluded because of the possible complicating factor, catamenia. The blood was drawn under sterile conditions after a fast of fifteen hours. It was divided into two parts, and an equal quantity placed in each of two sterile oxalate tubes. It was taken immediately to the laboratory where the sample was centrifuged at 30,000 r.p.m. for fifteen minutes, the plasma removed, and the first cholesterol estimation started within a half hour after the withdrawal of the blood. One tube of blood was kept at room temperature (21° to 24° C.) and the other at icebox temperature (8° C.). Subsequent cholesterol estimations were made on samples of blood drawn from these two specimens at one-, two-, four-, six-, eight-, and twenty-four-hour intervals. The method of determination of the cholesterol was adapted from Bloor,[6] where 1 c.c. of plasma was added slowly with shaking to approximately 40 c.c. of an alcohol-ether mixture (3:1) in a 50 c.c. volumetric flask. This was brought to the boiling point on a water bath, allowed to cool to room temperature, diluted to the 50 c.c. mark, and filtered into an Erlenmeyer flask through fat-free filter paper, the funnel being constantly covered to prevent evaporation. Twenty cubic centimeters of this filtrate were evaporated just to dryness at a temperature of 60° to 70° C. The residue was extracted three times with hot chloroform, using first 5 c.c., then 3 c.c., then 2 c.c. of chloroform, and transferring each portion to a 10 c.c. graduated, stoppered cylinder. The extractions were made up to 5 c.c. with

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TABLE I

SHOWING DETAILED VARIATIONS IN CHOLESTEROL VALUES AS MEASURED IN MG. PER 100 C.C. PLASMA IN EACH INDIVIDUAL FOR EACH TIME PERIOD, AND THE GAIN OR LOSS FOR EIGHT- AND TWENTY-FOUR-HOUR PERIODS

CASE	TIME IN HOURS							GAIN OR LOSS	
	½	1	2	4	6	8	24	8 HR.	24 HR.
Icebox Temperature Group									
1	129.5	128.3	122.5	122.0	122.0	119.6	121.4	- 9.9	- 8.1
2	132.5	136.3	133.7	133.7	132.5	126.3	122.0	- 6.2	-10.2
3	156.2	156.2	166.2	151.2	161.2	155.0	151.2	- 1.2	- 5.0
4	168.7	171.2	186.2	153.7	180.0	180.0	180.0	+ 11.3	+11.3
5	120.1	123.8	120.1	121.0	120.1	122.5	109.6	+ 2.4	-10.5
6	181.2	181.2	182.5	181.2	176.2	182.5	173.7	+ 1.3	- 7.5
7	200.0	200.0	196.2	200.0	200.0	192.5	185.0	- 7.5	-15.0
8	211.5	213.7	215.0	213.7	213.0	205.0	200.0	- 6.5	-11.5
9	160.2	158.7	165.0	165.0	156.0	153.7	147.6	- 6.5	-12.6
10	130.2	130.2	129.5	131.6	131.2	123.7	118.7	- 6.5	-11.5
11	136.2	133.7	133.7	133.7	133.7	147.5	147.5	+ 11.3	+11.3
12	148.7	147.5	150.0	147.5	147.5	167.5	188.7	+ 18.8	+40.0
13	165.0	165.0	161.2	142.5	166.2	165.0	151.2	± 0	-13.8
14	187.5	187.5	186.2	188.7	185.0	186.2	165.0	- 1.3	-22.5
15	123.7	122.5	123.7	121.7	122.5	118.7	116.2	- 5.0	- 7.5
16	188.7	187.5	186.2	187.5	188.7	192.5	191.2	+ 3.8	+ 2.5
17	156.2	156.2	170.0	172.5	170.0	168.7	168.7	+ 12.5	+12.5
18	203.7	205.0	240.0	245.0	245.0	250.0	245.0	+ 46.3	+41.3
19	165.0	165.0	165.0	163.7	165.0	165.0	163.7	± 0	- 1.3
20	200.0	200.0	198.7	200.0	200.0	245.0	255.0	+ 45.0	+55.0
21	161.2	161.2	160.0	163.7	161.2	155.0	148.7	- 6.2	-12.5
22	192.5	195.0	193.7	192.5	192.5	190.0	177.5	- 2.5	-15.0
23	188.5	188.5	187.5	185.0	188.7	198.7	200.0	+ 10.2	+11.5
24	196.2	196.2	211.2	205.0	206.2	205.0	217.5	+ 8.8	+21.3
25	151.2	152.5	152.5	153.7	155.0	151.2	161.2	± 0	+10.0
26	185.0	185.0	185.0	186.7	185.0	185.0	183.0	± 0	- 2.0
Total	4339.4	4347.9	4421.5	4361.5	4404.3	4451.8	4389.2	+112.4	+50.2
Avg.	166.9	167.2	170.5	167.7	169.3	171.2	168.8	+ 4.3	+ 1.9
Room Temperature Group									
1	129.5	126.9	118.3	115.3	123.1	120.1	120.7	- 9.4	- 8.8
2	132.5	131.2	135.5	133.7	132.5	126.3	122.0	- 6.2	-10.5
3	156.2	155.0	155.0	156.2	166.2	160.0	147.5	+ 3.8	- 8.7
4	168.7	168.7	180.0	156.2	153.7	180.0	180.0	+ 11.3	+11.3
5	120.1	124.5	121.3	124.5	124.5	130.0	113.1	+ 9.9	- 7.0
6	181.2	181.2	186.2	187.5	193.7	178.7	167.2	- 2.5	-14.0
7	200.0	200.0	200.0	200.0	201.7	203.7	187.5	+ 3.7	-12.5
8	211.5	213.7	213.7	213.7	213.7	205.0	200.0	- 6.5	-11.5
9	160.2	160.2	160.2	158.7	160.2	150.0	148.7	- 10.2	-11.5
10	130.2	130.2	127.5	131.6	128.7	127.5	116.2	- 2.7	-14.0
11	136.2	135.0	132.5	140.0	135.0	147.5	148.7	+ 11.3	+12.5
12	148.7	150.0	150.0	150.0	150.0	163.7	165.0	+ 15.0	+16.3
13	165.0	165.0	166.2	166.2	161.2	165.0	153.7	± 0	-11.3
14	187.5	187.5	187.5	187.5	187.5	165.0	166.2	- 22.5	-21.3
15	123.7	123.7	123.7	123.7	122.5	118.7	116.2	- 5.0	- 7.5
16	188.7	192.5	188.7	188.7	188.7	188.7	188.7	± 0	± 0
17	156.2	156.2	170.0	168.7	168.0	167.5	168.7	+ 11.3	+12.5
18	203.7	203.7	240.0	240.0	240.0	250.0	250.0	+ 46.3	+46.3
19	165.0	165.0	165.0	165.0	165.0	200.0	200.0	+ 35.0	+35.0
20	200.0	198.7	201.2	203.7	201.2	255.0	271.2	+ 55.0	+71.2
21	161.2	160.0	161.2	165.0	161.2	155.0	142.5	- 6.2	-18.7
22	192.5	191.2	192.5	190.0	190.0	192.5	178.7	± 0	-13.8
23	188.7	188.7	187.5	187.5	188.7	198.7	196.2	+ 10.0	+ 7.5
24	196.2	196.2	200.0	205.0	206.2	208.7	218.7	+ 12.5	+22.5
25	151.2	150.0	152.5	153.7	155.0	160.0	162.5	+ 8.8	+11.3
26	185.0	185.0	185.0	185.0	185.0	183.7	183.0	- 1.3	- 2.0
Total	4339.6	4340.0	4401.2	4397.1	4403.2	4501.0	4412.9	+161.4	+73.3
Avg.	166.9	166.9	169.2	169.1	169.3	173.1	169.7	+ 6.2	+ 2.819

TABLE II

SHOWING RANGE OF GROUP VALUES AND AVERAGE OF GROUP VALUES FOR EACH TIME PERIOD THE CHOLESTEROL WAS MEASURED, AND AVERAGE GAIN OR LOSS AND RANGE OF GAINS OR LOSSES FOR ENTIRE GROUP FOR EIGHT- AND TWENTY-FOUR-HOUR TIME PERIODS

TIME (HOURS)	RANGE	AVERAGE	AVERAGE GAIN OR LOSS	RANGE GAIN OR LOSS
Icebox Temperature Group				
½	120-211	166.9		
1	122-213	167.2		
2	120-240	170.0		
4	121-245	167.7		
6	120-245	169.3		
8	118-250	171.2	+4.7	+46.3 to - 9.9
24	109-255	168.8	+1.9	+41.3 to -22.5
Room Temperature Group				
½	120-211	166.9		
1	123-213	166.9		
2	118-240	169.2		
4	115-240	169.1		
6	122-240	169.3		
8	118-255	173.1	+6.2	+55.0 to -22.5
24	113-271	169.7	+2.8	+71.2 to -21.3

chloroform. In a similar cylinder 5 c.c. of the chloroform-cholesterol standard containing 0.1 mg. of cholesterol was pipetted. To both standard and unknown there was added 2.0 c.c. of acetic anhydride and 0.1 c.c. of concentrated sulfuric acid. Each cylinder was stoppered and mixed and permitted to stand in the light of the colorimeter for twenty-five minutes. The resulting yellow-green colors were compared in the colorimeter, and the cholesterol per 100 c.c. of plasma was calculated from the formula:

$$\frac{\text{Reading of Standard}}{\text{Reading of Unknown}} \times 125 = \text{mg. cholesterol per 100 c.c. of plasma.}$$

Each estimation was done in duplicate and the average value recorded in each instance. The error of the method was considered to be ± 5 mg.

The normal controls were the 21 cholesterolysis studies on normal individuals previously reported.

RESULTS

The total number of men in the study was 26. Their age range was from 21 to 33 years.

Table I shows the detailed cholesterol values in each individual for each time period as well as eight- and twenty-four-hour gain or loss.

Table II shows the range of gain or loss and the average value of cholesterol for the entire group for each time period.

From these tables it can be seen that the nine conclusions drawn for the normal group[4] alone equally hold true for the mentally abnormal group alone; but when the normal group is compared with the mentally abnormal group, the following differences are noted:

1. The average rate of change of blood cholesterol at room temperature and icebox temperature of the mentally abnormal group and of the normal group is experimentally identical.

2. At the end of eight and twenty-four hours the average cholesterol change of normal group is less than that of mentally ill group both at room temperature and at icebox temperature, although the difference is very small.

3. At the end of eight and twenty-four hours the range of gains for the icebox group is practically the same, but the range of losses is markedly less in the mentally abnormal group than in the mentally normal group. In the room temperature group the range of eight-hour gain is definitely greater, but the difference in the losses is slight; in the twenty-four-hour period the range of gain is definitely greater and the range of loss definitely less.

4. The curve of average cholesterol values of the abnormal group parallels that of the normal group throughout the twenty-four-hour period, but is always significantly below it, both in the icebox group and in the room temperature group. This is illustrated in Chart 1.

5. The range of gains or losses for the eight- and twenty-four-hour periods shows a significant shift in a tendency for the abnormal group to show gains over losses whereas this is not so for the normal group. This is illustrated in Chart 2.

DISCUSSION

In an earlier paper on cholesterolysis we pointed out that the problem of the rate of change of cholesterol in a specimen of blood is undoubtedly a complicated one, the result depending entirely upon the direction of equilibrium established by the involved factors: (a) the attempt of a cholesterol esterase to change free cholesterol to a combined form; (b) simple dissolution of cholesterol molecules by hydrolysis; (c) the types of anticoagulant used; and (d) the rate at which the cholesterol aggregates are able to free themselves from the meshes of protein molecules. These factors were thoroughly discussed at that time and to date our work does not present any contradictory material in this respect. The factor (d) then, as now, was the most intriguing, and there are additional studies relating to it which are worthy of presentation. Bellis and Scott[7] and Lehman and Scott,[8] while working with blood, reported a protein shift from cells to plasma following the addition of isosmotic dextrose, sodium chloride, calcium chloride, or barium chloride to oxalated or defibrinated blood in vitro. This shift caused an increase in the plasma-protein concentration which was proportional to the degree of dilution. The protein chiefly affected was albumin. Sandor[9] noticed that of the proteins of the blood the albumin fraction frequently contained lipids, of which one was cholesterol, and that the amount of lipid bound to the albumin was fairly constant regardless of the species or the individuals within it. On the other hand, he did not find this to be true of the globulin fraction, for here he found that the quantity of lipid bound to the globulin varied widely in different species and in different individuals within the same species. Moreover, he found that strikingly specific variations existed in the strength of the protein-lipoid bonds, for different alcohol concentrations were required to attain the maximum extraction of serum lipids. He suggested that lipids and proteins are linked through accessory valances rather than enmeshment. Went and Kúthy,[10] in their

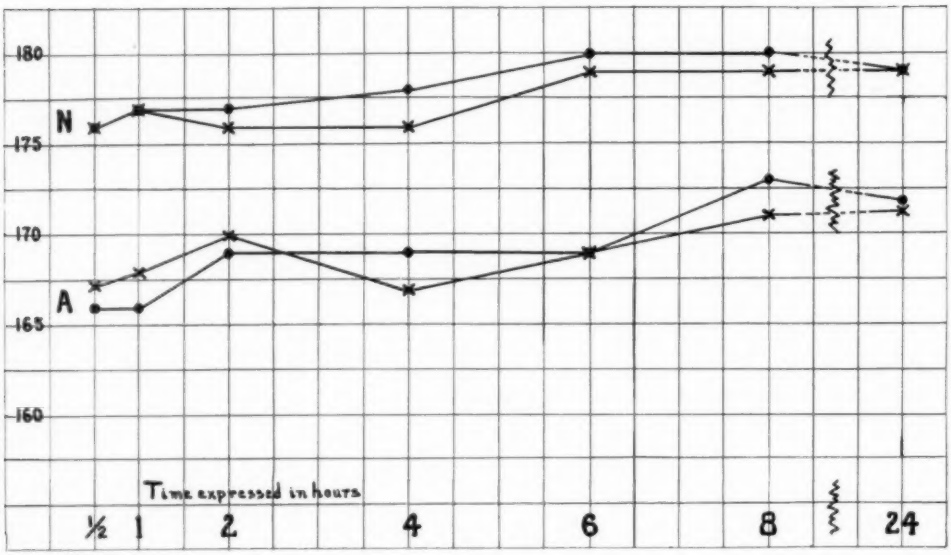

Chart 1.

A=Mentally ill group.
N=Normal control group.
●=Average room temperature values.
X=Average icebox temperature values.
Vertical figures=Milligrams cholesterol per 100 c.c. blood plasma.

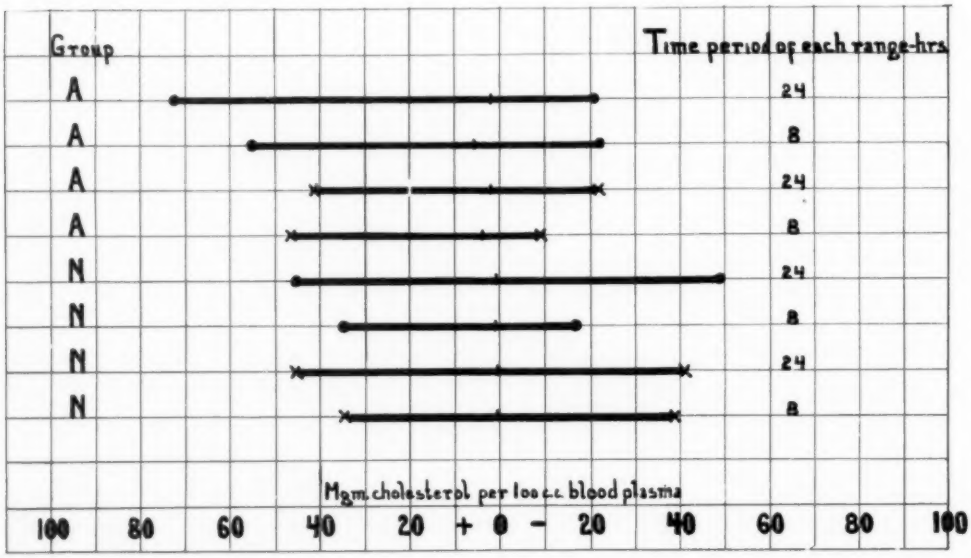

Chart 2.

A=Mentally ill group.
N=Normal control group.
●=Range room temperature values.
X=Range icebox temperature values.

studies of lipoid-protein complexes, found that these were easily formed by the addition of emulsified lecithin to native serum, but not to sera previously treated with alcohol-ether mixture. Süllmann and Verzar,[11] using lipemic blood, found that the lipids would diffuse through membranes in considerable amounts if they used membranes which were at least permeable to Congo red (pore diameter calculated to be 18 by 10^{-8} cm.). They discussed the possibility that unsplit protein-lipid complexes might be able to go through capillary walls, at least under special conditions, and feel that this is substantiated by the rough parallelism which appears to exist between the lipid and protein contents of some pathologic fluids. In confirmation of this Bruger[12] reported that in the course of the ultrafiltration of pleural and ascitic transudates, the cholesterol and proteins are concentrated to about the same extent in the ultrafiltration residue. He feels, though, that since cholesterol, but not the proteins, is adsorbed by a Berkefeld candle or by kieselguhr, the bond between the two substances must be a very weak one. Bang[13] long ago first noticed that the precipitation of globulin in the blood carried down with it a portion of the cholesterol, and he suggested at that time that the cholesterol was combined with the euglobulin fraction of the blood protein. Handovsky and Lohmann[14] carried this work farther and have reported that the higher the euglobulin in the blood the less the cholesterol that could be shaken out with ether. They found that 25 per cent of the cholesterol in ox serum was bound with the globulin. Gardner and Gainsborough,[15] likewise studying lipoid-protein complexes, reported that the percentage of the total plasma sterol retained in the proteins was 16.4; that euglobulin retained 13.8 per cent; pseudoglobulin 1.03 per cent; and albumin 1.5 per cent. Theorell,[16] while discussing the physical-chemical aspects of the blood lipids, stated that he felt the cholesterol to be held in combination not only with globulin but also with fibrinogen, and that at the isoelectric point of globulin all of the cholesterol can be extracted with ether; that fibrinogen holds the cholesterol firmly except at pH 6. Macheboeuf,[17] while separating compounds from the blood plasma containing both proteins and fatty substances, found that he was able to obtain an albumin fraction very rich in lecithin and cholesterol esters and which was also soluble in neutral or alkaline water to a clear solution containing up to 5 per cent.

These studies establish the following facts relative to factor (d) in the stability of cholesterol in blood plasma: (1) There is a state of protein equilibrium in the blood which can be upset in either direction by interference with the electrolyte balance of the blood. (2) There is a certain quantity of cholesterol adherent to the protein molecule. (3) The largest percentage of cholesterol adherent to the protein is adherent to the euglobulin fraction. (4) The bonds between the cholesterol and the euglobulin are fragile. (5) This bond may be of the nature of enmeshment, accessory valences, or adsorption. It can readily be seen from this that the presence or absence or direction of cholesterolysis in the blood plasma can, and probably does, depend upon the protein molecules therein.

The material evaluated here makes it only too clear that there are at work in the blood plasma of man some such complicated factors as the foregoing,

factors which make group evaluation of cholesterolysis impracticable and which in themselves insist upon individual attention. It is impossible to attribute such individual fluctuations as those observed to any simple variable such as age, sex, weight, or body type, for analysis in this respect only leads to a confusion of the issue. From a metabolic point of view, however, our results are interesting for two reasons: (1) the changes which occur in the plasma cholesterol of individuals with abnormal mental states are practically identical in nature with those of normal individuals, and (2) these changes always average upon a lower metabolic plane. Whether or not these facts are of any significance in mental disorders, it is difficult to state; that they conform with much other physical-chemical data which have accumulated relative to metabolism in neuropsychiatric cases is unquestionably true, for there is a definite body of literature indicating that in mental disorders, and particularly in dementia praecox, a lowered metabolic state exists and that this state closely parallels on a lower plane that of the normal individual. The reasons for this are, unfortunately, unknown, but they are undoubtedly based upon the physical-chemical limitations of the individual cells composing the body, the abnormal metabolism, as the abnormal mental symptomatology, being merely an indicator of abnormal cell function, the reasons for which have to be left to future research. The part which cholesterol plays in this metabolism is not at all clear, for all of our knowledge concerning its function in the metabolism of the individual is indirect and not at all conclusive. That it plays an important part is unquestioned; just what that part is must be left to future research.

SUMMARY

A study of the rate of change of total cholesterol in the blood plasma of mentally ill persons over a period of twenty-four hours is presented. Changes do occur in positive and negative directions which are similar to those of normal individuals but on a lower plane. It is very probable that these are not significant for the group, but they may be for the individual case.

REFERENCES

1. Schube, P. G.: Blood Cholesterol Studies in Mental Disease, Am. J. Psychiat. **12**: 355, 1932.
2. Schube, P. G.: Blood Cholesterol Studies in Mental Disease. II. Schizophrenia, Am. J. Psychiat. **12**: 1227, 1933.
3. Schube, P. G.: Blood Cholesterol and the Manic Depressive Psychosis, J. LAB. & CLIN. MED. **22**: 240, 1936.
4. Schube, P. G., Raskin, N., and Campbell, E.: Cholesterolysis in the Blood Plasma of Normal Man, J. LAB. & CLIN. MED. **23**: 711, 1938.
5. Schube, P. G.: Variations in the Blood Cholesterol of Man Over a Time Period, J. LAB. & CLIN. MED. **22**: 280, 1936.
6. Bloor, W. R.: Cholesterol in Blood, J. Biol. Chem. **24**: 277, 1916.
7. Bellis, C. J., and Scott, F. H.: Alteration of Protein Distribution in Vitro, Between Corpuscles and Plasma Caused by Isosmotic and Hyperosmotic Solutions, J. Biol. Chem. **111**: 17, 1935.
8. Lehman, W., and Scott, F.: Note on Total Protein Content of Plasma and Serum, J. Biol. Chem. **111**: 43, 1935.
9. Sandor, G.: Le probleme des proteides, Paris, 1934, Gaston Doin et Cie.
10. Went, S., and Kúthy, S.: Ueber die Rolle der Serumlipoide; physikalisch-chemische Beiträge, Ztschr. f. Immunitätsforsch. u. exper. Therap. **82**: 392, 1934.
11. Süllmann, H., and Verzar, F.: Diffusibilitat des Blutfettes, Biochem. Ztschr. **270**: 44, 1934.

12. Bruger, M.: Relation Between Cholesterol Partition and Total Protein Content in Pathological Body Fluids. State of Cholesterol in Such Fluids, J. Biol. Chem. **105**: 13, 1934.
13. Bang: Quoted by Handovsky and Lohmann.
14. Handovsky, H., and Lohmann, K.: Constitution of Blood Serum and Its Significance for Action of Poisons; Method for Determination of Cholesterol in Blood Serum, Arch. f. d. ges. Physiol. **210**: 59, 1925.
15. Gardner, J. A., and Gainsborough, H.: Studies on Cholesterol Content of Normal Human Plasma: Attraction of Proteins of Plasma for Sterols, Biochem. J. **21**: 141, 1927.
16. Theorell, H.: Studien über die Plasmalipoide des Blutes, Biochem. Ztschr. **223**: 1, 1930.
17. Machebœuf, M.: Recherches sur les phosphoammolipides et les stérides du sérum et du plasma sanguins; entraînement des phospholipides, des stérols et des stérides par les diverses fractions au cours du fractionnement des protéides du sérum, Bull. Soc. chim. biol. **11**: 268, 1929.

VITAMIN A DEFICIENCY: ITS PREVALENCE AND IMPORTANCE AS SHOWN BY A NEW TEST*

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THE importance of vitamin A in human nutrition is not settled. Overardent claims by some have been met with protests by others. The only specific disease commonly associated with vitamin A, xerophthalmia, is so rarely seen that some experts doubt the likelihood of a vitamin A deficiency being common. Recently instruments for testing subnormal dark adaptation have been used as indicators of vitamin A deficiency, but these have been criticised from a clinical viewpoint.

This paper presents (1) details of a new rapid visual test; (2) evidence of the test's relation to vitamin A; (3) evidence of the prevalence and clinical significance of vitamin A deficiency.

I. A RAPID VISUAL TEST

It is supposed that the recovery of vision after blinding by a bright light depends on a reformation of the retinal visual purple from vitamin A. Reviews connecting this process with night blindness, dark adaptation, visual purple, etc., by Adams,[1] Tansley,[2] Hecht,[3] Jeghers,[4] and Booher,[5] may be consulted. Various instruments for testing dark adaptation have been used, including Aubert's glowing wire of 1865,[6] the Birch-Hirschfeld photometer,[7] and various recent instruments by Tschernig,[8] Edmund,[9] Feldman,[10] Ferree and Rand,[11] and Jeans and Zentmire,[12] who call their instrument a "biophotometer."

The biophotometer, being available commercially, has dominated the recent reports in this field, and with the "visual adaptometer" of Hecht,[13] and the "adaptometer" of Feldman,[23] seems to be the chief instrument being used. These instruments are complicated in construction, seem to require practiced

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subjects for best results, and above all, require ten minutes or more for one test. Furthermore, severe criticisms, especially regarding the relation of these tests to vitamin A deficiency, have been advanced by Snelling,[14] Palmer and Blumberg,[15] Gridgeman and Wilkinson,[16] Isaacs, Jung, and Ivy.[17]

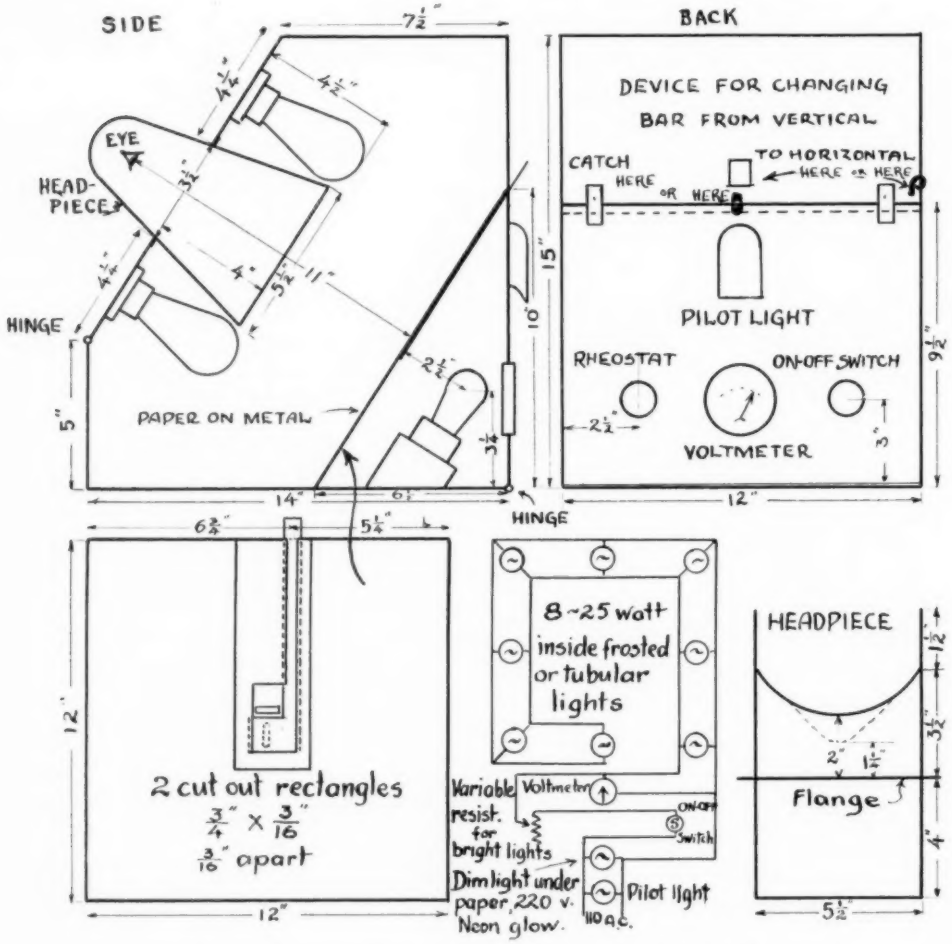

Fig. 1.—Drawings for one model of the apparatus described.

Description of Apparatus.—The instrument here outlined consists of a black metal (or wooden) box, about 1 cubic foot in volume. Protruding from the front is a headpiece, and on the back are various instruments, as seen in Fig. 1. Inside the box are: (1) A source of bright white light for bleaching visual purple in the eyes, coming from either one or several bulbs. (2) A surface for reflecting this bright light into the eyes, while looking into the headpiece, such that 50 foot-candles is the intensity at the eyes; the bright light is connected to a voltmeter and rheostat, and each instrument is calibrated by a photometer to operate at a certain voltage; the reflecting surface is paper (English Cartridge Paper, 100 pounds), is 12 inches square, and covers a metal shield from which two rectangles $\frac{3}{4}$ inch by $\frac{3}{16}$ inch are cut out. (3) Behind

the metal shield is a dim light, so placed as to shine evenly through the two rectangles. In passing through the paper this light loses 90 per cent of its intensity and at the eye has an intensity of about 4 millifoot-candles, and is slightly reddish. A mechanical device controlled from the back of the box is used to cover one of the rectangles of dim light, while the other remains visible, the subject then having to distinguish between vertical and horizontal directions.

Description of a Test.—The subject adjusts his head comfortably against the rubber forehead rest, wearing glasses if they are usually worn, and is shown the rectangles of dim light, one "Up," one "Across." The bright light is put on, and he is asked to look at a black spot marking the position where the bar of dim light will later appear when the bright light goes out. During this period of thirty seconds, the name, date, etc., can be recorded on a card and the voltage checked. As the watch hand crosses thirty seconds the bright light is turned off and the subject reminded to say "Up" or "Across," indicating the direction of the rectangle seen, as soon as the direction can be made out. The time is noted when this is said, and represents the recovery time from thirty seconds of standard bright light.

Tests are repeated at minute intervals, three usually sufficing. In some cases the three values may be rather divergent, especially when the first value is long. It is then considered more accurate to use the median or midvalue, rather than the mean; e.g., of three values 23, 15, 16, use 16 rather than 18. The range of recovery times is from three to sixty seconds, with a mode at eight. Thirty to 40 persons an hour have been tested by one operator with one instrument.

Discussion of Apparatus.—Previous investigations in this field have been hampered by instruments too slow and complicated to permit the testing and retesting of large numbers of people, thereby lacking statistical weight for their findings. Hence the insistence on a test that would be rapid and simple. Every step in the development of this design was tested on numerous subjects not for the accuracy with which it would show new laws of optic physiology, but rather for the avoidance of complications in practice when testing for vitamin A deficiency. Repeated tests were made on each person at each stage, permitting calculations of means, and their standard errors, thus giving mathematical assurance of the validity of every modification. The result is a nice balance, obtained by experimentation, of numerous factors, including the color, duration, and intensity of the bright light, the size, color, duration, and intensity of the dim light, and the area and location of the retina affected and other points.

Reflection of the bright light from paper makes it more diffuse than reflection from or transmission through glass. Diffusion of the light helps to avoid the afterglow in the eyes from bright spots of light that causes some confusion otherwise. Confusion may also be lessened by having the outer edges of the reflecting surface actually, but not apparently, brighter than the center where the dim light is to be seen. This is done by having a circle of lights for bright illumination.

Having the dim light in the form of a small rectangle which may be vertical or horizontal gives a more constant recovery time and eliminates guessing which

is common in children. A suitable size was found that would be independent of astigmatism in the subject. Glasses are worn, if they are usually worn, only for the sake of speed, since no effect of removing them has been found, even in extreme myopia and hyperopia. There must, of course, be light transmission and perception. The terms "Up" and "Across" are used because they are much more familiar to the average subject and much faster than any others tried.

Since the standard error of the mean has been found to be about one second for many people, checks are required only within about one second, and the "reaction times" of subject and operator are unimportant. A practice effect—tests gradually getting shorter—has sometimes been observed if more than three tests were made at one-minute intervals, but if the interval was lengthened to five or ten minutes this effect disappeared.

The relatively large error permitted prevents significance being attached to various factors that might influence the test. The afterglow has already been mentioned, but it has too small an influence. The rubber forehead rest cuts out enough light so that the room need not be darkened, provided no light is shining from directly behind the subject. The fixation point (black spot) holds the light on the fovea centralis, but the area of retina covered is about 3°, so rods are involved as well as cones, and the results are satisfactory. Blue or gray eyes admit more light to the fundus than brown and might be more affected by the bright light, but with the accuracy demanded no relation to eye color has been found. Similarly, age, sex, and fatigue seem not to affect this test.

Other factors not related to vitamin A might show an effect, but in general they do not. The size of pupil would have an effect if the light reflex is defective. Experiments on several people with homatropine in the eyes showed a much prolonged recovery time (five to ten times normal) with widely dilated pupils. Measurement of pupil size, as it returned to normal, showed a linear correlation with the recovery time. Full contraction of the pupil with pilocarpine showed no effect. Marked differences between the two eyes and lack of coordination may influence the values, particularly by making great variations in successive tests. Experimentally covering one eye doubles or trebles the time taken when the two eyes were used, though commonly both eyes gave the same individual value.

II. RELATION OF THE TEST TO VITAMIN A

Distribution of "Recovery Times." What Is "Normal"?—The frequency distribution of 1,519 people is shown in Fig. 2. The pronounced dip at eleven seconds suggests that this is a bimodal curve, which means a combination of two curves, each with its own mode. The two curves would be the distribution of (a) people "normal" in vitamin A and (b) people "deficient" in the vitamin, and are suggested by the broken lines. Assuming that the most common value (mode) of eight seconds is the mode of the distribution curve for normals, how can one decide the dividing line? An arbitrary standard has to be set up, just as all workers in this field have set up arbitrary points. In the beginning of

this work it was decided that any person with a "recovery time" of 11 or more seconds would be considered "deficient" in vitamin A, and that the deficiency would be greater as the time was longer. This arbitrary division is still used, though it errs in some cases, and receives support from studies of the response of vitamin A deficient people to vitamin A therapy.

Any person who does not show a change of recovery time after one dose of 50,000 I. U. of vitamin A, and who has no evidence of vitamin A deficiency is now considered "normal" in vitamin A. If a response is shown, it always consists of a shortened recovery time to a point where further treatment produces no effect. Such a person is considered "cured," in the absence of other signs of vitamin A deficiency. Hundreds of people have been so treated, and the work is being continued. Some have responded, some have not, and there is a surprisingly sharp division at the originally arbitrary time of eleven seconds.

Seasonal Variation.—A large group of students has been retested at intervals during the winter. About 500 students, equally divided into "normals" and "deficients" in their October tests, were involved. In the January retests of students who were normal in October, 26 per cent remained about the same value, 26 per cent had longer values (slightly deficient), while 48 per cent became definitely deficient (over 10 seconds). Of students who were deficient in vitamin A in October, 21 per cent became normal (mostly by treatment), 24 per cent remained about the same, and 55 per cent became more deficient. These figures show a clear tendency for adults to become more deficient as the winter goes on.

Evidence of the Test's Relation to Vitamin A.—The relation of this test to vitamin A is supported by the following evidence: (1) Of 1,500 tested, of whom 780 were "deficient" in vitamin A by the above standards, some 200 were given vitamin A. All, except 3, showed a reduction in recovery time. (2) The recovery time of a normal person was lengthened by taking a vitamin A-free diet, then reduced by vitamin A treatment. (3) Clinical observations on 4 patients with cirrhotic liver disease, 14 diabetic persons and 12 persons with gastric ulcers on Sippy diets, suggest a relation between dietary vitamin A or provitamins and the test. (4) Considerable subjective evidence of vitamin A deficiency with long recovery times and of improvement accompanying reduction of recovery time has been adduced.

1. *Response to Vitamin A by Deficient Cases.*—Two curves in Fig. 2 show the results obtained with the 200 people treated. All these people were retested during treatment until a constant recovery time was obtained, though usually this took only a short time. Repeated tests on controls, who received a vitamin A-free oil, showed no change from day to day. In Fig. 2 the curve with square dots shows the distribution of the untreated cases, from seven to forty-four seconds, and the round dots show the distribution after receiving vitamin A. The latter curve is sharply distributed around a mode of eight seconds, and is almost perfectly symmetrical between four and twelve seconds. These people are by definition "normal" in vitamin A, and the fact that their distribution curve is symmetrical about eight, supports the contention concerning a "normal" part of the large curve shown.

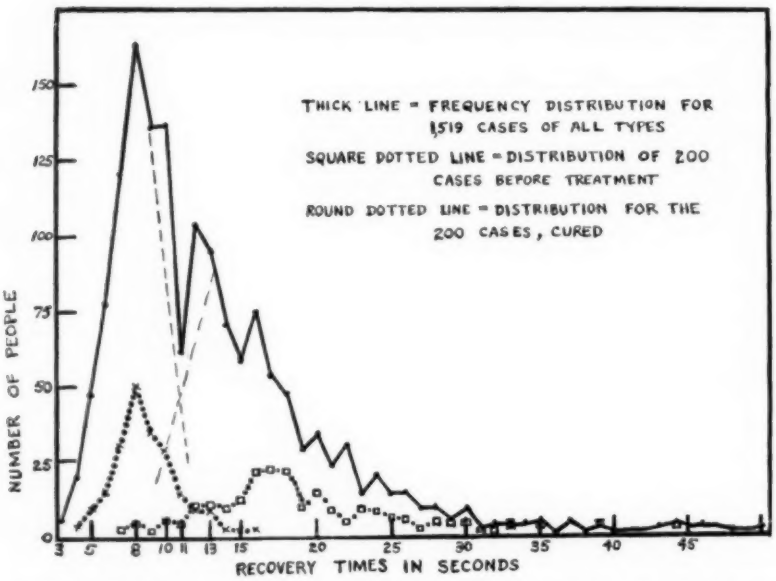
THICK LINE = FREQUENCY DISTRIBUTION FOR 1519 CASES OF ALL TYPES
SQUARE DOTTED LINE = DISTRIBUTION OF 200 CASES BEFORE TREATMENT
ROUND DOTTED LINE = DISTRIBUTION FOR THE 200 CASES, CURED
NUMBER OF PEOPLE
RECOVERY TIMES IN SECONDS

Fig. 2.—The number of people at each recovery time in this test. The dotted line shows the number of people cured by vitamin A at each time and is thus the distribution of normal values.

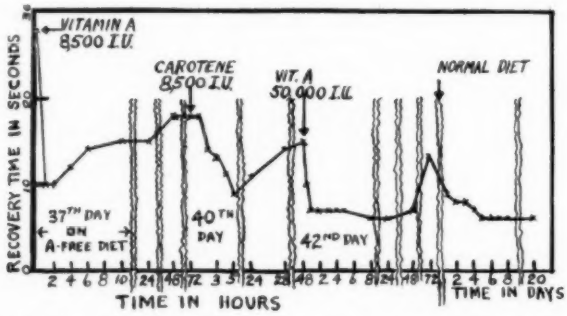

Fig. 3.—Time effects of small doses of vitamin A and carotene on the recovery times of a subject after thirty-seven days on a vitamin A-free diet. Increase in recovery time indicates the vitamin A deficiency.

Treatment and Dosage.—Treatment has been found most satisfactory when liver oil (usually halibut) of high vitamin A and low vitamin D content was used in capsules. Doses of 35,000 to 50,000 I. U. daily for three to five days usually resulted in a normal recovery time, and were much better than corresponding total dosages given 5 to 10,000 I. U. daily. People treated to this extent sometimes remained "normal" with no further supplement, but usually required about 5,000 I. U. daily for a further variable period of time, ascertained by tests. A number of people have been found who could only be maintained on doses of 10,000 I. U. daily or more. This is perhaps related to faulty absorption. In any case there has thus been established a wider individual variation and a higher daily requirement than has hitherto been described. Such people have invariably noted subjective improvement in various respects after the discovery of a high requirement and after subsequent treatment.

2. *Response of Normal Person to a Vitamin A Deficient Diet.*—An individual (myself) whose recovery time had been eight seconds for some months, and who had never experienced night blindness, eyestrain, dry skin, scanty saliva, or other signs of vitamin A deficiency, took a vitamin A-free diet for forty-five days. This diet was shown to contain less than 50 I. U. daily. The tests and coexistent symptoms are given in Table I for the first thirty-seven days, while Fig. 3 shows the effects of small doses of vitamin A or carotene for the remainder of the test period.

TABLE I

THE LENGTHENING OF THE RECOVERY TIMES OF INDIVIDUAL ON A VITAMIN A-FREE DIET, TOGETHER WITH ACCOMPANYING SYMPTOMS

DAYS ON VIT. A-FREE DIET	RECOVERY TIME SECONDS	COMMENTS
0	8	Same value daily for previous two weeks
1-9	8	
10-17	9	
18-22	11	Anorexia, which persisted two weeks
23-25	12	Mild diarrhea, persisted one week
26	14	Sore throat; rhinitis
27	15	
28, 29	16	"Cold" cleared up
30	17	Saliva noticeably decreased from now on
31, 32	18	Skin dry and scaling
33-35	20	Small gingival ulcers; night blindness
36	22	Eyes painful; no corneal ulcers
37	27	Epithelial casts in urine

The slow response shown in Table I, in which twenty-eight days were needed to reach a seriously deficient recovery time, may be explained on the basis of vitamin A and carotene storage in the liver. Moore[18] has shown that large reserves are carried even in a variety of serious diseases, and by calculation 1,000,000 I. U. may be a conservative estimate of the average person's reserve supply. It is not necessary to assume that this reserve must be exhausted before an effect be noted in a test, since some conservation may be expected. But work on experimental animals has shown that considerable depletion of liver carotene and vitamin A occurs before even the earliest metaplasia of epithelial tissues is observed (Bessey and Wolbach[19]).

As noted in Table I, signs of vitamin A deficiency were observed: anorexia, diarrhea, asialia, painful eyes, and photophobia, gingival ulcers, dry and scaling skin, nyctalo-hemeralopia, urinary epithelial casts.

Fig. 3 shows that treatment with only 8,500 I. U. of vitamin A brought intense salivation and immediate response in the recovery times. These returned to the normal eight-second level for two hours, but gradually increased to deficient levels, presumably as the vitamin was removed from the blood. An equivalent dose of carotene (kindly supplied by the S. M. A. Corp.) brought a slower response, requiring five hours to attain the same level. This might be due to slower absorption from the gut, or to the need of conversion into vitamin A by the liver before affecting the test. Absorption in both cases was complete, as shown by analyses of urine and feces. A dose of

50,000 I. U. of vitamin A resulted in a recovery time of six seconds (lower than the previous base level). Shortly afterwards a normal diet was resumed, and after some days a recovery time of six seconds was attained.

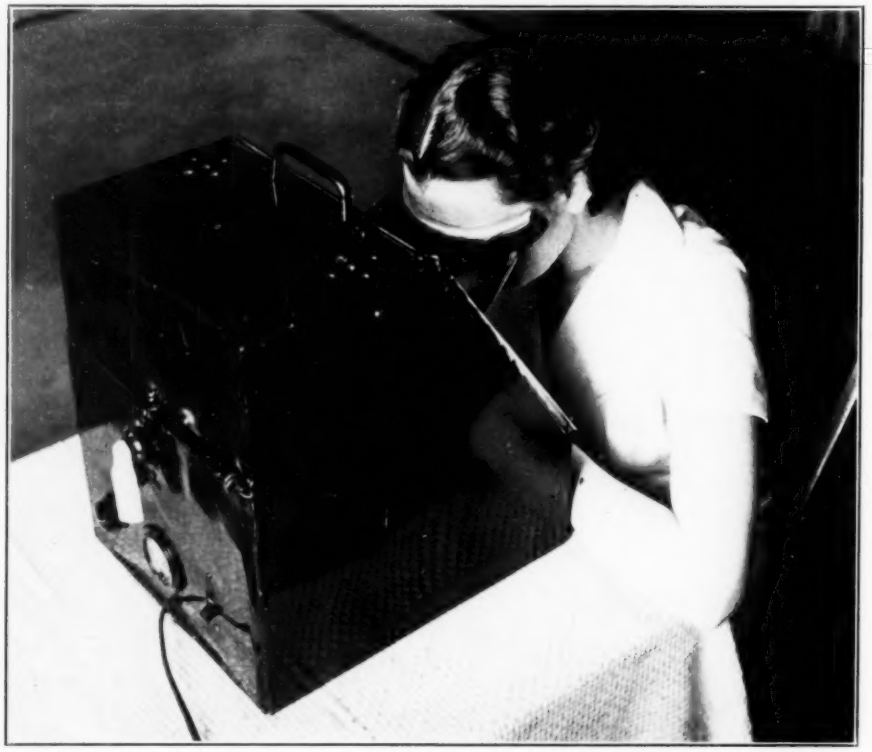

Fig. 4.

The disappearance of symptoms of vitamin A deficiency is of considerable interest. The salivation following intake of vitamin A on the thirty-seventh day of the vitamin A-free diet was over in twenty-four hours and marked the restoration of salivary flow, at first somewhat scarce. The painful eyes, which had been wrongly suspected of being due to corneal ulcers, were completely relieved on the thirty-ninth day. The gingival ulcers and night blindness seemed improved on the forty-second day, and were gone on the forty-fifth day. Scaling of the skin continued for about three weeks, and epithelial casts were found in the urine for five weeks.

Similar studies have been reported by Hecht and Mandelbaum,[20] and by Wald, Jeghers, and Arminio[21] but without any complete consideration of attendant symptoms.

3. *Clinical Evidence.*—(a) Liver disease. Haig, Hecht, and Patek[22] reported that 11 persons with cirrhotic liver disease had poor dark adaptation and were beneficially treated by large doses of vitamin A. These observations have been confirmed on 4 cases with values of twenty-two, twenty-seven, thirty-six, and fifty seconds. Presumably the conversion of carotenoids (provitamins) in the

livers of such people is impaired. Since the apparent vitamin A content of most diets is due to the carotenoids, impaired conversion leads to a deficiency even on an apparently adequate diet.

(b) Diabetic persons. Such a condition has also been found among diabetic persons where some liver dysfunction is usually present, and the diet contains large amounts of carotenoids. In 14 persons with diabetes, every case showed an apparent deficiency in vitamin A by this test, values ranging from thirteen to forty-five seconds. Further work is required on this new and interesting point.

(c) Gastric ulcers and special diets. A different type of vitamin A deficiency may be caused by special diets, restricting the intake of certain foods. Some of the most vitamin A-deficient cases encountered were found to be on Sippy or other ulcer diet, or on a diet for cholecystic disease. Values ranging from eleven to fifty-seven seconds were recorded. It seems not to have been realized that these diets are seriously deficient in vitamin A. In 12 ulcer cases specially treated with vitamin A concentrates more rapid improvement than expected was noted, and in one case a tendency to recurrence seems to have been checked. These observations must be extended before a full report can be made.

Such observations are in accord with the theory that lack of vitamin A may aggravate diseases in epithelial tissues (including mucous membranes) and may help their healing, without being directly responsible for the disease.

4. *Subjective Observations Related to Deficiency and Treatment.*—It is commonly claimed that these tests for dark adaptation, etc., provide the earliest clinical signs of a vitamin A deficiency, and it is tacitly assumed that a slight deficiency is a more or less serious condition which should be corrected. Few attempts have been made, however, to link these slight deficiencies with known clinical conditions. The most important clinical work has been done by Feldman,[23] but his study lacks statistical backing. The importance of poor vision at twilight (night blindness) in car accidents has recently been much emphasized.

A start on this problem of correlating symptoms with an objective test has been made by requiring students to answer certain questions at the time of being tested, and following up the answers later. A total of 753 such case histories have been summarized in Table II.

TABLE II

PERCENTAGE OF THE "NORMAL" GROUP (393 PERSONS) AND OF THE "DEFICIENT" GROUP (360 PERSONS) FOUND TO BE SUFFERING FROM VARIOUS CONDITIONS

CONDITION	PER CENT OF NORMALS	PER CENT OF DEFICIENTS
Consciously night blind	7	18
Eyestrain and photophobia	25	36
Dry prickly conjunctiva	5	10
Dry skin	7	17
Dry nasal mucosa	15	19
Scanty saliva (asialia)	2	6
Cold within a month of test	54	69
Average duration of colds	4 days	8 days

Many people found to be deficient in vitamin A were treated or treated themselves with various sources of vitamin A during several months. Most of these cases were retested, and often volunteered evidence of subjective improvement, even while unaware whether the test itself had shown improvement. This type of evidence is not capable of accurate expression, and involves so many psychic and other factors that it is commonly valueless. Nevertheless, some cases have been so striking as to command attention, and one such case will be described briefly.

A local airport attendant had to send up nightly a small meteorologic balloon with a dim light attached to it, and then follow its progress with a telescope. Sometimes he could not see the balloon for some time. On examination he was found to be "deficient" in vitamin A. Treatment brought forth obvious improvement in his ability to spot the balloon, as well as a shortened recovery time. It is likely that many around an airport are so affected by the beacons and ground lights that they cannot see dim lights quickly. There may even be greater demands on their system for vitamin A.

In any case it is clear that the test here described provides a rapid means for distinguishing people who are unadapted to some kinds of work, or who are working inefficiently, all depending on the recovery time from bright lights.

III. PREVALENCE OF VITAMIN A DEFICIENCY

The original studies of Jeans and Zentmire[24] showed a vitamin A deficiency among children as high as 56 per cent. Similarly, Maitra and Harris[25] have reported studies on various groups of children with a deficiency averaging about 30 per cent for the 193 studied. Both these reports present a limited number of cases and attempt to draw conclusions on small groups of 20 and 30, owing to the inherent slowness and complication of the instruments used. Nevertheless, the chief arguments at the present time concern the probable extent of vitamin A deficiency, and the results on a large number of people are, therefore, especially important. Table III presents the results for different classes and ages of people so far studied. This type of work is now being extended.

TABLE III

PERCENTAGE OF PERSONS IN VARIOUS GROUPS FOUND TO BE DEFICIENT IN VITAMIN A BY THE TEST REPORTED IN THIS PAPER

GROUP	TOTAL NUMBER	PER CENT DEFICIENT
All cases to date	1603	52
Children (ages 8-15)	69	49
Freshmen (ages 17-20)	225	51
Freshettes	130	52
Other university students (ages 18-23)	640	52
Single men on relief (ages 20-35)	90	64
Other adults (over 25)	449	53

No special comment on the table is necessary except to note that the per cent deficiency in this district—52 per cent—seems a little higher than reported elsewhere. It can also be observed that age and sex made no significant difference to this figure, but that men on relief showed a higher percentage. The latter figure requires more extended observations before comment.

SUMMARY

1. Details are given of a rapid visual test for vitamin A deficiency, depending on the recovery time after looking at a bright light. One test takes less than one minute, three tests being done within 5 minutes of the subject entering the room. This rapidity is valuable where large groups of people are concerned. The application to airmen, motorists, and the like, is pointed out.

2. Proof of the test's relation to vitamin A has been given by: (a) a report of experimentally induced vitamin A deficiency, correlating the test with other signs; (b) the prolonged recovery times observed in people with liver disease (cirrhosis or diabetes) or on special diets, whereby they either do not receive the vitamin or do not convert it from carotenoids; (c) the recovery on treatment with vitamin A of some 200 cases classed as deficient; and (d) subjective improvement in many such cases.

3. The effects on the test of sex, age, color of eyes, myopia, hyperopia, astigmatism, wearing of spectacles, and fatigue are negligible, since great accuracy is not demanded. The size of pupil, disparity of vision of the two eyes, and diseases might have an effect. These points are discussed.

4. Of 1,600 people examined 52 per cent have been found deficient in vitamin A, the incidence being higher among unemployed.

5. A table is given showing a higher incidence among vitamin A deficient people of night blindness, eyestrain, dry conjunctiva, dry skin, scanty saliva, and frequency and duration of colds.

6. Adults tended to become more deficient in vitamin A in February than they were in October.

It is unfortunately impossible to mention all the people who have helped in these investigations, either by patience in being tested, or by discussions. Special thanks for cooperation are due to M. R. Levey, M.D., C.M., D.L.O., of the Department of Ophthalmology; to D. E. Smith, Ph.D., of the Department of Psychology; to M. M. Cantor, B.S., M.D.; to A. McKeever and to R. Clelland.

REFERENCES

1. Adams, D.: Dark Adaptation: A Review, Medical Research Council, Special Report Series No. 127, 1929.
2. Tansley, K.: The Regeneration of Visual Purple. Its Relation to Dark Adaptation and Night Blindness, J. Physiol. **71**: 442, 1931.
3. Hecht, S.: Rods, Cones and the Chemical Basis of Vision, Physiol. Rev. **17**: 239, 1937.
4. Jeghers, H.: Night Blindness as a Criterion of Vitamin A Deficiency, Ann. Int. Med. **10**: 1304, 1937.
5. Booher, L. E.: Vitamin A Requirements, J. A. M. A. **110**: 1920, 1938.
6. Aubert, H.: Physiologie der Netzhaut, Breslau, 1865.
7. Birch-Hirschfeld, A.: Ueber Nachtblindheit im Kriege, Arch. f. ophth. **92**: 18, 1916.
8. Tschernig, M.: On the Question of the Adaptation of Vision, Acta ophth. **1**: 215, 1924.
9. Edmund, C.: Some Methods of Measuring Dark Vision, Acta ophth. **3**: 153, 1925.
10. Feldman, J. B.: Instrument for Determining the Course of Dark Adaptation, Arch. ophth. **12**: 81, 1934; **17**: 668, 1937.
11. Ferree, C. E., and Rand, G.: The Testing of Fitness for Night Flying: Light Sense, Am. J. Ophth. **20**: 797, 1937.
12. Jeans, P. C., and Zentmire, Z.: A Clinical Method for Determining Moderate Degrees of Vitamin A Deficiency, J. A. M. A. **102**: 892, 1934; Jeans, P. C., Blanchard, E., and Zentmire, Z.: Dark Adaptation and Vitamin A., J. A. M. A. **108**: 451, 1937.
13. Hecht, S.: Dark Adaptation and the Diagnosis of Avitaminosis A. Nutrition: The Newer Diagnostic Methods, New York, 1938, Milbank Memorial Fund.
14. Snelling, C. E.: A Study of the Birch-Hirschfeld Photometric Test for Vitamin A Deficiency, J. Pediat. **9**: 655, 1936.

15. Palmer, C. E., and Blumberg, H.: The Use of a Dark Adaptation Technic (Biophotometer) in the Measurement of Vitamin A Deficiency in Children, Pub. Health Rep. **52**: 1403, 1937.
16. Gridgeman, N. T., and Wilkinson, H.: Night Blindness and Vitamin A Deficiency. The Use of the Biophotometer, Lancet **234**: 905, 1938.
17. Isaacs, B. L., Jung, F. T., and Ivy, A. C.: Vitamin A Deficiency and Dark Adaptation, J. A. M. A. **111**: 777, 1938.
18. Moore, T.: The Vitamin A Reserves of the Adult Human Being in Health and Disease, Biochem. J. **31**: 155, 1937.
19. Bessey, O. A., and Wolbach, S. B.: Vitamin A Physiology and Pathology, J. A. M. A. **110**: 2072, 1938.
20. Hecht, S., and Mandelbaum, J.: Rod-Cone Adaptation and Vitamin A, Science **88**: 219, 1938.
21. Wald, G., Jeghers, H., and Arminio, J.: An Experiment in Human Dietary Night Blindness, Am. J. Physiol. **123**: 732, 1938.
22. Haig, C., Hecht, S., and Patek, A. J., Jr.: Vitamin A and Rod-Cone Dark Adaptation in Cirrhosis of the Liver, Science **87**: 534, 1938.
23. Feldman, J. B.: The Use of the Photometer in Detecting Latent Avitaminosis A. Nutrition: The Newer Diagnostic Methods, New York, 1938, Milbank Memorial Fund.
24. Jeans, P. C., and Zentmire, Z.: The Prevalence of Vitamin A Deficiency Among Iowa School Children, J. A. M. A. **106**: 996, 1936.
25. Maitra, M. K., and Harris, L. J.: Vitamin A Deficiency Among School Children in London and Cambridge, Lancet **233**: 1009, 1937.

THE EFFECT OF HYPOTHALAMIC LESIONS ON FEVER INDUCED BY INTRAVENOUS INJECTION OF TYPHOID-PARATYPHOID VACCINE*

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THE current conception of the nervous regulation of body temperature during the course of a disease like malaria is that, preceding the chill and period of rising temperature, the temperature-regulating mechanism suddenly becomes set at a higher level, with resultant increased heat production (shivering) and decreased heat elimination (vasoconstriction). The fall in temperature occurs when the regulating mechanism again becomes set at a lower level (DuBois[1]). It is only within the last few years that the location of the temperature-regulating mechanism within the hypothalamus has become sufficiently well known to make a satisfactory experimental approach to this subject possible, and the experiments which have been reported in the literature have not contributed much to its elucidation.

The reactions of animals with various lesions of the central nervous system to pyrogenic agents have been described by numerous investigators (among others, Freund and Strasmann;[2] Citron and Leschke;[3] Isenschmid;[4] Freund and Grafe;[5] Balcar, Sansum, and Woodyatt;[6] Girndt;[7] Solari;[8] Keller and Hare;[9] Thauer[10]). From many of these papers it is difficult to draw definite conclu-

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sions. Frequently acute animals were used, often in a state of shock; usually not more than two to ten days were allowed for recovery from operation. Protocols are often incomplete, and microscopic verification of the extent of the lesions is lacking. In most cases it is not apparent that adequate care was taken to stabilize the animal's temperature before exhibition of the pyrogenic agent. Dosages were usually varied from animal to animal.

Citron and Leschke,[3] using rabbits, made sections of the medial hypothalamus at the anterior border of the midbrain. Such animals did not react with fever to injections of *Trypanosoma nagana*, staphylococci, colloidal suspensions of paraffin, or even to beta-tetrahydronaphthylamine, but they were apparently in severe postoperative shock. Isenschmid[4] found that midbrain animals or animals with cervical cord transection responded with temperature rises to injection of sodium salicylate and to beta-tetrahydronaphthylamine after their body temperatures had been previously stabilized in an incubator.

Pinkston, Bard, and Rioch[11] injected typhoid-paratyphoid vaccine intravenously into a dog with chronic bilateral ablation of the neopallium which spared the hypothalamus. The result was an essentially normal fever curve.

Girndt[7] found that many rabbits from which he had removed the greater portion of the cortex bilaterally, responded to injection of *B. coli* vaccine with temperature falls instead of rises; but that if the cortex were removed on only one side dorsally, the temperature response to *B. coli* vaccine was normal. It would not be surprising if these extensive decortications had produced considerable hypothalamic damage. Unfortunately, there was no histologic verification of the extent of the lesions. In animals with a second type of operation (unilateral, circumscribed, dorsal decortication), he obtained a normal febrile response; then, without anesthesia, he pushed a blunt cannula into the medial hypothalamus through the free space dorsally and through it injected various drugs to test their central antipyretic effect. The mere introduction of the cannula and injection through it of normal saline solution produced an irreversible hypothermia in several animals previously inoculated with *B. coli* vaccine; in many others it resulted in milder temperature falls. These observations are of special interest because of the important part which the hypothalamus is known to play in temperature regulation.

In the present investigation an attempt has been made to determine the effect of hypothalamic lesions in cats on the reaction of these animals to the intravenous injection of typhoid-paratyphoid vaccine.

METHODS

Electrolytic lesions of the hypothalamus (or prechiasmatic region in several cases) were made with the Horsley-Clarke stereotaxic instrument in 21 cats. The exact location of each lesion was determined by serial sections through the hypothalamus stained alternately by Weil's method and by cresyl violet. The ability of each cat to regulate its body temperature was studied in considerable detail both preoperatively and postoperatively by means of numerous tests in both hot and cold boxes.

Sixteen normal cats were subjected to intravenous injection of typhoid-paratyphoid vaccine to establish the normal response. Each of the 21 operated cats, after a convalescence of a month or more, was subjected to exactly the same procedure.

Each cat was brought to the laboratory about 8 A.M. and placed on a specially designed canvas animal board to which it was strapped comfortably but securely. The body temperature was recorded as a continuous curve by the ink-writer of a Leeds and Northrup resistance temperature recorder, the resistance unit of which was inserted through the rectum to a constant measured distance of 14 cm. from the anal orifice. An area 3 to 4 cm. in diameter on the side of the chest had been depilated the previous day, and to this area a skin thermocouple was applied by a spring-mounted holder mounted on a ring stand. The room temperature and humidity were read at frequent intervals from a wet and dry bulb thermometer ventilated at about 3 meters per second by an electric fan.* In each case all the apparatus was mounted and set in function as soon as the cat was brought to the laboratory, and the resistance recorder traced a curve of the body temperature until this had become completely stabilized (often several hours). All temperatures are recorded in degrees Fahrenheit. After the temperature stabilization was accomplished, 0.35 c.c. per kg. of typhoid-paratyphoid vaccine was injected into the saphenous vein. This vaccine was all poured at one time from one freshly prepared stock bottle, kindly furnished by the Abbott Laboratories, Inc. It contained 1,000 million killed typhoid organisms, 750 million killed paratyphoid alpha, and 750 million killed paratyphoid beta organisms in each cubic centimeter. The dosage was not varied at any time. About 5 P.M. the skin thermocouple was dismounted and the fan ventilating the wet bulb thermometer was stopped. The setup was left otherwise undisturbed, tracing a constant record of the animal's temperature until the next morning, about twenty hours after injection.

In order to determine whether lesions in other parts of the brain would have a similar effect on the reaction of cats to typhoid-paratyphoid vaccine another series of experiments was made later but with a different lot of vaccine. This second lot was as nearly as possible of the same potency as the first, but it is not possible to be certain that the two series of experiments are altogether comparable.

THE NORMAL RESPONSE

The normal cat responds to intravenous injection of typhoid-paratyphoid vaccine with a cyclic response in which first the heat-preservation mechanism, then the heat-loss mechanism predominates. The periods of dominance of the heat preservation and elaboration mechanism are accompanied by shivering, slowing of the respiratory rate, and signs of generalized sympathetic stimulation, such as pupillary dilatation, cutaneous vasoconstriction, and erection of hair. The periods during which the heat-loss mechanism predominates are accompanied by marked acceleration in respiratory rate, by cessation of shivering, and by signs of generalized parasympathetic stimulation, such as pupillary constric-

*The maximal room temperature variation during the course of any one experiment was 2° F.; the maximal variation in relative humidity during any one experiment was 2 per cent.

tion, defecatory movements, urination, penile erection, and cutaneous vasodilatation; during such periods the temperature falls.

Usually immediately, almost always within ten to thirty minutes after injection, the temperature begins to rise, accompanied by shivering and signs of generalized sympathetic activity; it reaches a first temperature peak averaging 1.2° F. above the stabilized base line at an average interval of forty-eight minutes after the injection (Fig. 1). Shivering then ceases, respiration accelerates sharply, and under signs of marked generalized parasympathetic stimulation the body temperature drops sharply to a first intermediate low point which averages

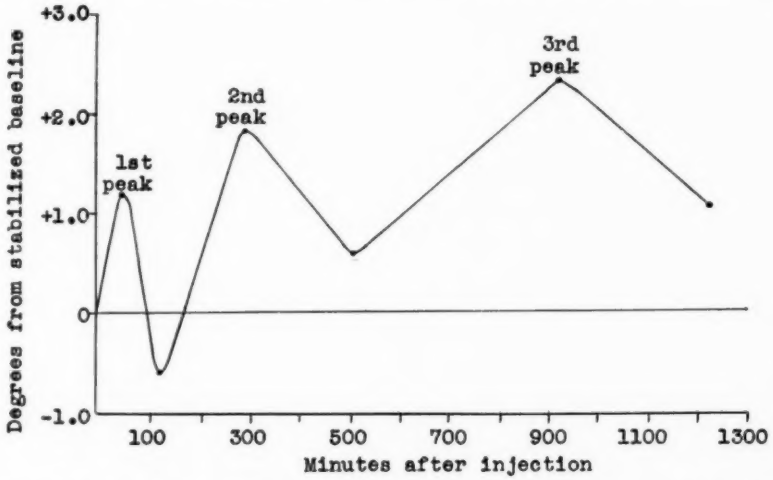

Fig. 1.—Composite fever curve, 16 normal cats, after injection of typhoid-paratyphoid vaccine intravenously.

0.6° F. below the base line, reached on an average about two hours after injection. Now the animal begins to shiver, sympathetic activity again becomes predominant, and the temperature rises to a second peak, averaging +1.8° F., reached on an average four and one-half hours after injection. Again the temperature falls, reaching another low point, averaging +0.57° F., at an average eight and one-half hours after the injection, rising then to a third peak which averages +2.3° F., fifteen and one-half hours after the injection. The temperature falls away slowly from this third peak but it is still well above the base line the next morning, averaging +1.06°, twenty hours after the injection.

The extreme range of variation in the normal was represented by the normal cat of Experiment 22, which, after holding its temperature practically constant during the first forty-five minutes after injection, showed a rapid drop in body temperature thereafter, the temperature reaching a low point of 2.3° F. below the base line, three and one-half hours after the injection. From this point, however, the temperature rose, reaching 0.4° F. above the base line eighteen hours and forty minutes after the injection; at the termination of the experiment twenty-one hours after the injection the body temperaure was exactly at the base line. The next most extreme variation from the average in the normal series of cats was shown by the normal cat of Experiment 26. During the first forty minutes this cat's temperature remained practically stationary, falling

only slightly. Then the temperature fell markedly, reaching a low point of 3.5° below the base line, one hour and forty-three minutes after the injection. From this low point, however, the temperature rose rapidly, reaching a high point of 2.8° F. above the base line, sixteen hours after the injection. The next morning, nineteen hours after the injection, the cat's temperature was still 1.2° F. above the base line.

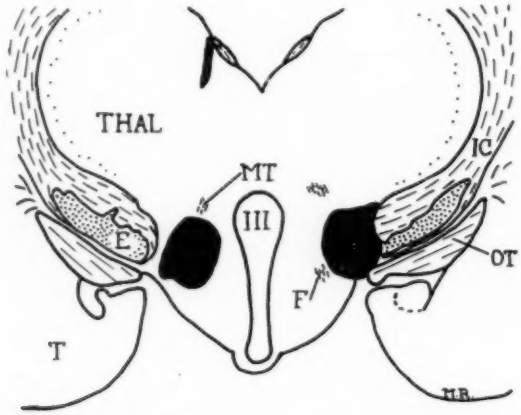

Fig. 2.—A diagrammatic drawing of a frontal section through the diencephalon of Cat 20 with bilaterally symmetrical lesions laterally placed in the rostral part of the hypothalamus. *F*, fornix; *IC*, internal capsule; *MT*, mammillothalamic tract; *OT*, optic tract; *T*, temporal lobe; *THAL*, thalamus; *III*, third ventricle.

RESPONSE OF CATS WITH HYPOTHALAMIC LESIONS

Cats with certain hypothalamic lesions, on the other hand, show strikingly different temperature curves after intravenous injection of typhoid-paratyphoid vaccine. The temperature falls many degrees below the stabilized base line, and the hypothermia persists so that the next morning at the termination of the experiment the temperature is sill depressed 4° F. or more.

In this series of 21 cats with lesions of the hypothalamus (or in a few cases of the prechiasmatic region) there was not a single case which showed a deviation of the febrile response in the direction of an increase in the height or steepness of slope of the fever curve. Only one animal showed a typical normal fever curve. Six cats reacted to the vaccine with fever curves, which while falling within the range of the more atypical normal reactions to the vaccine, showed a definite deviation in the direction of smaller than usual rises and greater than average falls. Two animals responded to the injection of the vaccine with moderate falls in temperature. The rest of the series, 12 cats in all, showed marked and prolonged temperature falls which were well beyond any deviations from the average encountered in our normal series of cats.

Cat 20 will serve as an example. Bilateral lesions were placed in the lateral hypothalamus, as illustrated in Fig. 2. There resulted a slight impairment of ability to regulate against cold and a marked and prolonged loss of ability to regulate against heat. As late as sixty-seven days after the operation the cat failed to pant or sweat when it was placed in a hot box, although its rectal temperature was raised to 106°. It was injected with typhoid-paratyphoid vaccine

fifty-six days after the operation, and the marked and prolonged drop in temperature shown in Fig. 3 occurred. Seven days after the injection of the vaccine we injected into the same cat 20 mg. per kg. of alpha 1-2-4-dinitrophenol intravenously (3 per cent solution in sterile water containing 1.5 per cent sodium bicarbonate), the technique of the experiment being otherwise the same as when vaccine was injected. The temperature rose 3.4° in three and one-half hours and remained steady at about that level until the termination of the experiment, 5 hours after the injection, at which time the animal's temperature was 3.3° above the base line. Whatever it may have been that was responsible for the fall in temperature following the injection of the vaccine did not prevent dinitrophenol from producing fever.

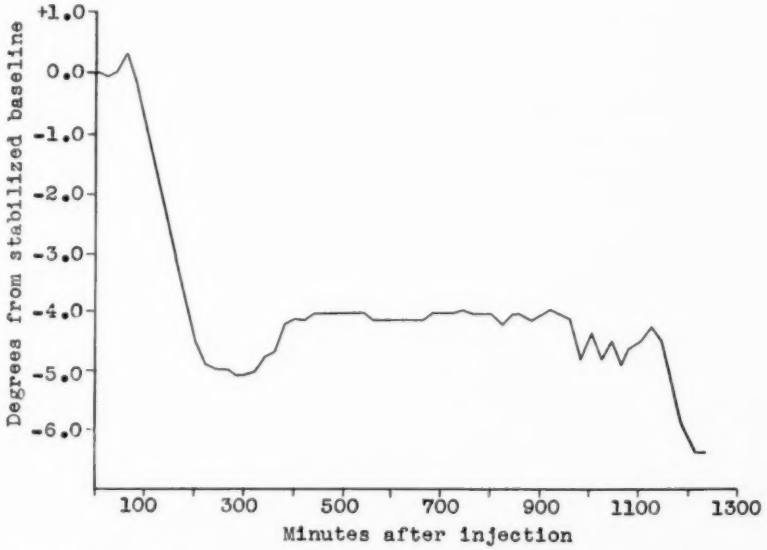

Fig. 3.—Chart showing marked fall of temperature in Cat 20 following intravenous injection of typhoid-paratyphoid vaccine.

In the period of the first fall of temperature after injection of the vaccine (fall from the first peak), the operated animals reacting with hypothermia showed an average rise in skin temperature due to vasodilatation of 1.5°, whereas those animals reacting with essentially normal curves showed an average rise due to vasodilatation of 1°. The corresponding figures for our normal, unoperated cats was 0.4°.

In the period of the first fall (fall from the first temperature peak), the average maximum respiratory rate attained by the cats reacting with hypothermia was higher than that attained by the animals responding with approximately normal curves (2.3 times the stabilized preinjection respiratory rate against 1.7 times; an absolute increase in respiratory rate of 50.3 per minute against 28 per minute). Shivering was about as often seen in those animals that reacted to the injection with hypothermia as in those that reacted with approximately normal fever curves. Eight out of 12 of the animals, reacting with hypothermia, shivered during the course of the experiment; 4 out of 6 of

the animals, reacting with fairly normal curves, shivered during the corresponding period of time. In a series of normal unoperated cats, 14 out of 16 shivered during the same period.

In those animals which responded to the injection of the vaccine with hypothermia, there was very often during the first period of thirty to forty minutes pupillary constriction, defecation, and salivation; occasionally the pupils were of medium size, seldom dilated. Something contained in the vaccine, therefore, stimulated the heat-loss mechanism, causing not only acceleration of respiration but also vasodilatation along with signs of parasympathetic activation.

In the second series marked falls in temperature resulted from injection of typhoid-paratyphoid vaccine in 1 out of 4 cats from which the frontal lobes had been removed and in 2 out of 10 cats with lesions in the dorsal thalamus. Similar reaction to the vaccine was obtained from an undernourished but otherwise normal cat. This cat had been kept on an inadequate diet, a small quantity of milk with plenty of water, for seventy-six days until it had lost 49.8 per cent of its original body weight and had then been given a full diet for seventeen days and at the time of injection had reached 72 per cent of its original weight. Two unoperated cats which were sick with distemper also gave drops instead of rises in temperature as a result of vaccine injection. These observations tend to throw doubt on the specificity of the reactions obtained from the cats with hypothalamic lesions. It is possible that several factors may be responsible for these abnormal reactions. But the fact that in the first series of experiments 12 out of 21 cats with hypothalamic lesions showed marked and prolonged temperature falls which were well beyond any deviations from the average encountered in the normal series, cannot be too lightly thrust aside. While a few of the 12 operated cats which showed marked drops in temperature were poorly nourished, the majority of them were in excellent condition, well nourished, and free from any obvious infection.

While there is a pronounced difference between the results obtained from cats with hypothalamic lesions and from those of the normal series, this difference must be interpreted cautiously. The drops in temperature in the cats with hypothalamic lesions did not appear to be dependent on damage to the hypothalamic heat-regulating centers and occurred in some cats in which these lesions had caused little or no disturbance in temperature regulation. It did not appear to be important whether these lesions were medially or laterally, rostrally or caudally placed. It is true that in the 6 cats with lesions in the caudolateral part of the hypothalamus, which showed markedly impaired ability to regulate against cold, 5 showed marked falls in temperature following the injection of typhoid-paratyphoid vaccine and only one showed a normal fever curve. This one cat and 2 others, i.e., 3 of the 6, belonged to the second series of experiments. But just as marked falls were obtained in some other cats in which there was no obvious disturbance in temperature regulation.

DISCUSSION

In a paper dealing with the thermal reactions induced by the intravenous injection of typhoid-paratyphoid vaccine in normal cats (Ranson, Jr.[12]), evi-

dence was presented which seems to show that the vaccine stimulates both the mechanism for conservation of bodily heat, including the sympathetic system, and the mechanism for heat loss, including the parasympathetic system. There is a great deal of evidence that the hypothalamus serves as a general sympathetic center and that its destruction interferes with heat production and conservation. The present investigation was begun with the idea of studying the effect on febrile reactions of the destruction of this center. It soon became obvious, however, that typhoid-paratyphoid vaccine which had been chosen as the fever-producing agent does not always cause a typical fever in normal cats and that its action is influenced by a number of obscure and uncontrollable factors. Because of this difficulty the investigation has not yielded as much information as might be desired in regard to the role of the hypothalamus in fever. Whether it does or does not play a part remains undetermined. The frequent occurrence of marked drops in temperature instead of rises in cats with hypothalamic lesions might be interpreted in favor of such a role, but, on the other hand, the absence of any correlation between the amount of disturbance in temperature regulation caused by the lesions and the character of the temperature curve following typhoid-paratyphoid injection may be taken as evidence to the contrary. Furthermore, in one cat of the second series, in which the lesions were placed bilaterally in the caudolateral part of the hypothalamus and produced a marked impairment in ability to regulate both against heat and cold, the vaccine caused a fairly normal fever reaction with a rise in temperature of 3°. If this experiment may be taken at its face value it would seem to indicate that the vaccine may cause fever by stimulating the heat-production and heat-conservation mechanism at a point below the hypothalamus.

One point, however, is clear. The marked falls in temperature, which occur in some cats following injection of typhoid-paratyphoid vaccine, are not due to the stimulation of that part of the heat-loss mechanism which lies in the hypothalamus and preoptic region. It has been shown that the receptive part of the mechanism lies in the preoptic region, that it is activated by moderate rises in temperature, and that a pathway leads backward from it through the lateral part of the hypothalamus to a heat-loss mechanism located behind the hypothalamus. When this descending pathway from the anteriorly placed heat-sensitive center is interrupted by bilaterally symmetrical lesions in the lateral part of the hypothalamus, the animal loses its ability to protect itself against overheating (Magoun, Harrison, Brobeck and Ranson;[13] Clark, Magoun and Ranson[14]). In 4 cats of the first series and one of the second, in which the lesions had been so placed as to interrupt this descending path bilaterally and to prevent the animals from panting or sweating when the rectal temperature was raised to 106°, injection of typhoid-paratyphoid vaccine caused marked drops in temperature. This shows that the part of the heat-loss mechanism which is stimulated by the injection lies caudal to the hypothalamus.

SUMMARY

Of 21 cats with chronic lesions of the hypothalamus (and in a few cases, prechiasmatic region) there was not a single case which showed a deviation of the fever curve in response to the intravenous injection of typhoid-paratyphoid

vaccine in the direction of an increase in height or steepness of slope. Only one animal showed a typical normal fever curve. Twelve showed marked and prolonged temperature falls (3° to 11° F.) which lasted for a period of twenty or more hours.

Although such animals shivered normally in response to the injection of the vaccine, the temperature did not rise appreciably in response to such shivering; the pupillary dilatation which usually accompanies such periods of shivering and rising temperature in the normal animal was more often absent in these hypothalamic animals responding to the vaccine with hypothermia. Instead, there was pupillary constriction or the pupil was medium. There were often coincident signs of parasympathetic overactivity, such as defecation, urination, and salivation. The respiratory rate increased markedly, greatly exceeding that seen in the normal series of cats.

It is impossible at this time to make any definite anatomical localization for lesions resulting in this reaction. It does not appear to be important whether the lesions are laterally or medially, rostrally or caudally placed, or whether the lesion results in a disturbance in the normal regulation of body temperature.

A hypothalamic lesion which results in a hypothermic reaction to the injection of typhoid-paratyphoid vaccine intravenously does not prevent the animal from responding to the intravenous injection of alpha-dinitrophenol with a typical febrile response.

The part of the heat-loss mechanism which is activated by the intravenous injection of typhoid-paratyphoid vaccine does not lie in the hypothalamus but somewhere caudal to it.

REFERENCES

1. DuBois, E. F.: Basal Metabolism in Health and Disease, Philadelphia, 1936, Lea & Febiger.
2. Freund, H., and Strasmann, R.: Zur Kenntnis des nervösen Mechanismus der Wärmeregulation, Arch. f. exper. Path. u. Pharmakol. **69**: 12, 1912.
3. Citron, J., and Leschke, E.: Ueber den Einfluss der Ausschaltung des Zwischenhirns auf das infectiöse und nichtinfectiöse Fieber, Ztschr. f. exper. Path. u. Therap. **14**: 379, 1913.
4. Isenschmid, R.: Ueber die Wirkung der die Körpertemperatur beeinflussenden Gifte auf Tiere ohne Wärmeregulation, Arch. f. exper. Path. u. Pharmakol. **75**: 10, 1913.
5. Freund, H., and Grafe, E.: Über die Beeinflussung des Gesamtstoffwechsels und des Eiweissumsatzes beim Warmblüter durch operative Eingriffe am Zentralnervensystem, Arch. f. d. ges. Physiol. **168**: 1, 1917.
6. Balcar, J. O., Sansum, W. D., and Woodyatt, R. T.: Fever and the Water Reserve of the Body, Arch. Int. Med. **24**: 116, 1919.
7. Girndt, O.: Über den Angriffspunkt von Antipyreticis: Chinin und chininähnlichen Stoffen, Arch. f. exper. Path. u. Pharmakol. **140**: 91, 1929.
8. Solari, L. A.: Température et réactions thermiques chez les chiens hypophysoprives ou à tuber lésé, Compt. rend. Soc. de biol. **108**: 125, 1931.
9. Keller, A. D., and Hare, W. K.: Heat Regulation in Medullary and Midbrain Preparations, Proc. Soc. Exper. Biol. & Med. **29**: 1067, 1932.
10. Thauer, R.: Wärmeregulation und Fieberfähigkeit nach operativen Eingriffen am Nervensystem homoiothermer Säugetiere, Arch. f. d. ges. Physiol. **236**: 102, 1935.
11. Pinkston, J. O., Bard, P., and Rioch, D. McK.: Responses to Changes in Environmental Temperature After Removal of Portions of Forebrain, Am. J. Physiol. **109**: 515, 1934.
12. Ranson, S. W., Jr.: Fever Induced by the Intravenous Injection of Typhoid-Paratyphoid Vaccine, Arch. Int. Med. **61**: 285, 1938.
13. Magoun, H. W., Harrison, F., Brobeck, J. R., and Ranson, S. W.: Activation of Heat Loss Mechanisms by Local Heating of the Brain, J. Neurophysiol. **1**: 101, 1938.
14. Clark, George, Magoun, H. W., and Ranson, S. W.: Hypothalamic Regulation of Body Temperature, J. Neurophysiol. **2**: 61, 1939.

THE RELATIONSHIP OF THE INSULIN HYPOGLYCEMIC REACTION TO SHOCK*

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THE term *shock* is commonly applied loosely to various conditions of disease, quite diverse in character. Moon has segregated one definite entity from the heterogeneous group by the presence of a characteristic combination of physiologic features. He defined shock as a circulatory deficiency not cardiac and not vasomotor in origin, characterized by decreased blood volume, decreased volume flow, and hemoconcentration. He showed also that this syndrome is accompanied by characteristic pathologic changes seen post mortem. These include widespread capillovenous congestion and evidences of increased capillary permeability such as edema, effusions, and petechiae in visceral areas.

Hypoglycemic manifestations, resulting from the administration of insulin, are often spoken of as "insulin shock." It seems pertinent to determine whether this reaction has the characteristic features of shock as defined. This can be done by determining the presence or absence of hemoconcentration during "insulin shock" and by noting changes present in the viscera after death from large doses of insulin. Experiments on dogs and rabbits were arranged to secure data on these points.

The dogs employed were of both sexes and ranged from 8 to 15 kg. in weight. There were 4 white rabbits and 2 rabbits of the Dutch breed. These were all young adult males, apparently in perfect health and weighing approximately 2 kg. each. The variability in their insulin tolerance perhaps was related to a factor of sex. Dotti has reported that bucks are much less constant than does in their responses to insulin injections. All animals were maintained on standard kennel rations. A fasting period of eighteen hours preceded each of the experiments on dogs. The rabbits were not fasted, a fact which may have further added to the variability in their tolerance to insulin. Hemoglobin determinations, red blood cell counts, and determinations of the erythrocyte volume were made in all instances on venous blood. The blood was obtained from the jugular vein in the dog and from the marginal vein of the ear in the rabbit. In dogs the hemoglobin determinations were made by the Sheard-Sanford photelometer;[16] in rabbits the Haden-Hausser clinical hemoglobinometer was used. Van Allen hematocrit tubes were employed in determining the volume of erythrocytes.

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The dogs were divided into three groups:

Group 1: Three dogs received intravenous injections of 1 unit of insulin per kg. of body weight every two hours until convulsions occurred. They were then resuscitated by intravenous injections of glucose. The blood was examined at hourly intervals (Table I).

TABLE I

DOGS RECEIVING 1 UNIT OF INSULIN PER KILOGRAM OF BODY WEIGHT EVERY TWO HOURS UNTIL CONVULSIONS OCCURRED

TIME IN HOURS	0	1	2	3	4	5	6	7	8	9	10
					Dog 1						
R.B.C. in millions	8.05	8.05	8.50	7.75	8.00	7.50	7.10	8.50	8.25	7.90	8.30
Hemoglobin in grams	19.2	20.8	19.0	16.8	17.2	16.0	15.2	17.4	18.1	19.0	17.8
Hematocrit reading	52	56	54	52	50	46	50	46	51	49	48
W.B.C. in thousands	11.5	10.0	12.1	16.9	15.8	14.4	17.4	19.9	16.8	19.0	18.8
					Dog 2						
R.B.C. in millions	7.80	8.05	8.20	8.20	7.95	8.10	7.80	8.70			
Hemoglobin in grams	16.4	18.6	18.0	16.6	18.2	17.4	16.8	18.8			
Hematocrit reading	50	54	54	51	53	49	53	56			
W.B.C. in thousands	10.1	8.70	10.4	12.3	14.0	14.2	14.8	15.7			
					Dog 3						
R.B.C. in millions	7.91	8.50	8.10	7.95	7.55	7.13	7.05	8.35			
Hemoglobin in grams	17.2	17.8	17.6	16.8	16.6	17.2	15.2	15.8			
Hematocrit reading	51	55	53	52	48	51	47	48			
W.B.C. in thousands	10.8	13.2	17.3	23.3	18.6	20.2	16.4	16.2			

TABLE II

DOGS RECEIVING 2 UNITS OF INSULIN PER KILOGRAM OF BODY WEIGHT

(Convulsions occurred early because these animals had been subjected to insulin experiments on the previous day. See text.)

TIME IN HOURS	0	½	1	1½	2	2½
			Dog 4			
R.B.C. in millions	8.40	7.90	8.00	7.75	8.30	8.05
Hemoglobin in grams	18.0	17.6	18.2	17.5	18.0	17.6
Hematocrit reading	53.5	54.0	52.0	49.5	54.0	53.0
			Dog 5			
R.B.C. in millions	6.87	6.90	6.65			
Hemoglobin in grams	14.2	14.6	13.4			
Hematocrit reading	49.0	49.0	50.0			
			Dog 6			
R.B.C. in millions	7.04	7.20	6.80	7.05		
Hemoglobin in grams	17.4	17.0	14.8	16.6		
Hematocrit reading	55.0	53.0	52.0	53.5		

TABLE III

TWENTY UNITS OF INSULIN PER KILOGRAM OF BODY WEIGHT ADMINISTERED TO DOG 7; 25 PER KG. TO DOG 8

("C" indicates the time at which the first convulsion occurred; "D," the time at which death ensued.)

TIME IN HOURS	0	4	6	6½	7	7¼	8½	8¾	9	12	22	25
Dog 7												
R.B.C. in millions	7.15	7.50	C	7.00					7.65	7.20	7.30	D
Hemoglobin in grams	13.0	12.8	C	14.2					14.4	13.4	14.2	D
Hematocrit reading	44	48	C	43					46	44	46	D
Dog 8												
R.B.C. in millions	6.45	6.60			C	6.85	6.50	D				
Hemoglobin in grams	14.2	13.0			C	14.0	14.5	D				
Hematocrit reading	42	40			C	44	42	D				

TABLE IV

RABBIT 1 RECEIVED 20 UNITS OF INSULIN PER KILOGRAM OF BODY WEIGHT; RABBIT 2, 50 UNITS PER KG.; RABBIT 3, 75 UNITS PER KG.; AND RABBIT 4, 100 UNITS PER KG.

TIME IN HOURS	0	2	2½	3½	4¼	5	6	6½	6¾	8	9¼	9½	9¾
Rabbit 1													
R.B.C. in millions	5.85	C	5.75	5.50	D								
Hemoglobin in grams	11	C	11	11	D								
Hematocrit reading	32.5	C	34.5	33.5	D								
Rabbit 2													
R.B.C. in millions	5.85						C		6.10	6.05	5.90	D	
Hemoglobin in grams	11						C		12	12	11	D	
Hematocrit reading	34						C		33.5	33	34	D	
Rabbit 3													
R.B.C. in millions	4.85								C	5.30	5.00		D
Hemoglobin in grams	10								C	11	10		D
Hematocrit reading	30.5								C	30.0	30.0		D
Rabbit 4													
R.B.C. in millions	5.90			C	5.85	6.55		6.05	D				
Hemoglobin in grams	11			C	11.5	12.0		12.0	D				
Hematocrit reading	33.5			C	38.0	35.5		38.0	D				

Group 2: The three dogs in this group received 2 units of insulin per kg. of body weight intravenously (Table II). Convulsions occurred early in each of these dogs because on the previous day they had been subjected to repeated

small injections of insulin until convulsions occurred. Decrease in resistance to hypoglycemia on successive days of insulin administration has already been reported.[3] Blood samples were obtained on the animals of group 2 at half hourly intervals. Following the occurrence of convulsions, they too were resuscitated by injections of glucose.

Group 3: Table III summarizes the protocols of the two dogs of group 3. Large doses of insulin (20 to 25 units per kg., respectively) were given to these dogs subcutaneously. Several samples of blood were obtained after the onset of convulsions, which occurred at frequent intervals until death ensued. Post-mortem examinations were made on both of these animals. The viscera appeared normal in all respects. There were no evidences of dilatation and engorgement of the capillaries and venules. Neither petechial hemorrhages, edema, nor serous effusions were present in any of the viscera.

Four rabbits received single massive doses of insulin subcutaneously, and the concentration of their blood was determined at irregular intervals until death ensued (Table IV). Two rabbits were given nonfatal doses of insulin intravenously followed by larger doses twenty-four hours later. Similar blood studies were made on these animals (Table V). Post-mortem examinations were made on all of the rabbits immediately following death. Again there were no visceral evidences of capillovenous congestion or increased capillary permeability.

TABLE V

ANIMALS RECEIVING 10 UNITS OF INSULIN PER KILOGRAM OF BODY WEIGHT AT THE BEGINNING OF THE EXPERIMENT AND 20 UNITS PER KG. AT THE TWENTY-FOURTH HOUR

TIME IN HOURS	0	6	24	26	27¼	28	28½	28¾	29¾
Rabbit 5									
R.B.C. in millions	6.35	6.60	6.85	6.50	C	6.80	D		
Hemoglobin in grams	12	12	13	12	C	13	D		
Hematocrit reading	37.5	38.0	36.0	37.5	C	39.0	D		
Rabbit 6									
R.B.C. in millions	5.25	5.05	5.20	5.55		5.65	C	5.30	D
Hemoglobin in grams	10	10	11	10		11	C	10	D
Hematocrit reading	31.5	32.0	30.0	32.5		33.0	C	31.5	D

The data assembled in the accompanying tables indicate that in these experiments hemoconcentration did not result from the intravenous or subcutaneous injection of nonfatal or fatal doses of insulin. Post-mortem examination revealed none of the visceral changes found in animals after death by experimental shock.[13]

COMMENTS

Some data concerning hemoconcentration have been recorded in reports dealing with the effects of insulin. Drabkin and Edwards reported that large doses of insulin given to dogs were followed by anhydremia as indicated by an increased hemoglobin percentage. Levine and Kolars reported increased numbers of leucocytes and erythrocytes after injections of insulin in rabbits. They interpreted this increase as evidence of anhydremia. They did not state the dosage of insulin employed, but believed there was no relationship between the

size of the dose and the degree of anhydremia. Chaikelis made determinations of erythrocyte counts, the hemoglobin, and the water content of the blood of rabbits after small doses of insulin. He stated that a moderate anhydremia accompanied the hypoglycemia and confirmed the observation (Levine and Kolars) that the degree of anhydremia was unrelated to the dosage of insulin. Anhydremia during insulin hypoglycemia has been accepted to such an extent that many authors have incorporated it in theoretical explanations of various features of the insulin reaction.

Other reports are at variance with those cited. Mazzocco and Morera did not find increased numbers of erythrocytes during insulin hypoglycemia in dogs. Olmstead and Taylor found no significant change in the hemoglobin percentage after moderate doses of insulin in rabbits. In the dog an increase in the hemoglobin and the number and volume of erythrocytes may occur as the result of contraction of the spleen with subsequent emptying of its contents.[1, 6, 8, 17] However, in the rabbit[9] and in man, animals in which the spleen is comparatively smaller, less muscular, and much less active, hemoconcentration is almost entirely dependent upon loss of fluid from the vascular bed. Obviously this entails a decrease in the blood volume. Yet Kay and Smith found no changes in the blood volume of rabbits after the administration of insulin. Furthermore, Chaikelis concluded that the apparent decrease in the water content of the blood, which he reported in rabbits, could be shown to exist only by the statistical treatment of a large group of observations. He stated that the individual results were too varied and the degree of change was too small to be demonstrated by individual observations or even by short series of observations.

The data supplied by Drabkin and Edwards indicate that they actually were dealing with hemoconcentration. The conditions of their experiments were such that true shock may have originated from the combination of large doses of insulin, long anesthesia (injected intraperitoneally), and operative procedures. Indeed, in the one dog on which additional data were obtained, reductions of 53.1 per cent in the plasma volume, and of 42.1 per cent in the total blood volume were found. These findings indicate that shock, rather than hypoglycemia, was the major physiologic disturbance in these animals.

The increase in the number of leucocytes noted in Table I is in agreement with the findings of Levine and Kolars, Nitzescu and Mangiuca and others. Various explanations have been suggested for the occurrence of leucocytosis during insulin hypoglycemia. It probably results from the adrenalinemia consequent to the injection of insulin. That adrenalin causes lymphocytosis and neutrophilia, has been demonstrated by other investigators (Garrey and Bryan).

The absence of hemoconcentration and of post-mortem evidences of capillovenous congestion and increased capillary permeability indicate that the mechanism of death from large doses of insulin is not related to the mechanism of shock. Further evidence of the dissimilarity of the two mechanisms is the prompt recovery from the insulin reaction obtained by the administration of sugar. There are no available means by which such a dramatic recovery may be obtained in shock.

SUMMARY

1. Determinations of the hemoglobin content, and the number and volume of erythrocytes in the blood of dogs and rabbits, indicate that hemoconcentration does not occur incident to hypoglycemic reactions resulting from injections of nonfatal or fatal doses of insulin.

2. Visceral evidences of capillovenous congestion and increased capillary permeability were not seen in animals after death by insulin hypoglycemia.

3. The mechanism of death resulting from large doses of insulin is not the same as that of shock. The term "insulin shock" is confusing and should be abandoned.

4. The leucocytosis recorded in previous reports on insulin hypoglycemia was confirmed.

Since this article was written studies on the protein content and the colloid osmotic pressure of the serum in man and the dog during insulin hypoglycemia have been published by Butt and Keys (Arch. Int. Med. **63**: 156, 1939). They concluded that "'insulin shock' bears no close relation to other types of shock."

REFERENCES

1. Bollman, J. L., Svirbely, J. L., and Mann, F. C.: Blood Concentration Influenced by Ether and Amytal Anesthesia, Surgery **4**: 881, 1938.
2. Chaikelis, S. A.: The Effect of Insulin on the Glucose-Chloride Relationship and Anhydremia in the Blood of Rabbits, Thesis, Columbia Univ. Press, 1933.
3. Corwin, W. C.: Decreased Resistance to Hypoglycemia on Successive Days of Administration of Insulin, Am. J. Physiol. **125**: 227, 1939.
Preliminary Report (Corwin), Proc. Staff Meet., Mayo Clin. **12**: 716, 1937.
4. Dotti, L. B.: Response of the Rabbit to Insulin, Am. J. Physiol. **114**: 538, 1936.
5. Drabkin, D. L., and Edwards, D. L.: The Production of Anhydremia With Insulin, Am. J. Physiol. **70**: 273, 1924.
6. Essex, H. E., Seeley, S. F., Higgins, G. M., and Mann, F. C.: Effect of Ether Anaesthesia and Amytal Anaesthesia on the Erythrocytic Findings in Control and Splenectomized Dogs, Proc. Soc. Exper. Biol. & Med. **35**: 154, 1936.
7. Garrey, W. E., and Bryan, W. R.: Variations in White Blood Cell Counts, Physiol. Rev. **15**: 597, 1935.
8. Hausner, E., Essex, H. E., and Mann, F. C.: Roentgenologic Observations of the Spleen of the Dog Under Ether, Sodium Amytal, Pentobarbital Sodium and Pentothal Sodium Anesthesia, Am. J. Physiol. **121**: 387, 1938.
9. Higgins, G. M., and Corwin, W. C.: The Effect of Splenectomy on the Number of Erythrocytes and Leucocytes in the Peripheral Blood of Rats and Rabbits Under Ether and Sodium Amytal Anesthesia, Surgery **1**: 703, 1937.
10. Kay, H. D., and Smith, W.: The Effect of Insulin on the Blood Volume, J. Physiol. **59**: 80, 1924-25.
11. Levine, V. R., and Kolars, J. J.: The Effect of Insulin on the Morphological Blood Picture, Proc. Soc. Exper. Biol. & Med. **22**: 169, 1924.
12. Mazzocco, P., and Morera, V.: Accion de la insulina sobre la composicion de la sangre, Rev. Asoc. méd. argent. **37**: 60, 1924.
13. Moon, Virgil H.: Shock, Its Mechanism and Pathology, Arch. Path. **24**: 642, 794, 1937; Shock and Related Capillary Phenomena, New York, 1938, Oxford Univ. Press.
14. Nitzescu, I., and Mangiuca, I.: Action de l'insuline sur l'equilibre hemoglobulaire, Compt. rend. Soc. de Biol. **90**: 1347, 1924.
15. Olmstead, J. M. D., and Taylor, A. C.: The Effect of Insulin on the Blood, Am. J. Physiol. **69**: 142, 1924.
16. Sanford, A. H., Sheard, C., and Osterberg, A. E.: The Photelometer and Its Use in the Clinical Laboratory, Am. J. Clin. Path. **3**: 405, 1933.
17. Searles, P. W., and Essex, H. E.: Changes in Blood in the Course of Ether Anesthesia and Sodium Amytal Anesthesia, Proc. Staff Meet., Mayo Clin. **11**: 481, 1936.

LABORATORY METHODS

ADDITIONAL RECORDINGS OBTAINED WITH THE OSCILLATO-CAPACIGRAPH*

C. FENNING, M.D., AND B. E. BONAR,† M.D., SALT LAKE CITY, UTAH

RECENTLY Fenning[1] described an instrument capable of recording, simply and graphically, small changes in capacity. Previous publications by Bonar and Fenning[2] included records of respiratory movements in small animals, uterine contractions in rats and guinea pigs, fetal movements and fetal respiratory movements. Since these studies were made, additional recordings have been obtained which show other possible physiologic and clinical uses of the instrument.

As previously implied, the instrument may be used to detect and record on photosensitive paper any activity which directly or indirectly produces a displacement of the surface of the body under investigation. Provisions must be made for placement of the plate used in conjunction with the instrument in proximity to, but not in contact with, the surface under investigation.

With proper adjustment of the circuit of the instrument, extremely high sensitivity is available. Fig. 1 well illustrates this fact. This figure represents the apex beat displacement recorded from the anterior thoracic wall of a rat fetus weighing 0.85 gm. The actual displacement in this case was difficult to see with the unaided eye. Reflected light from the moist, glistening surface of the thorax provided the necessary amplification factor for recognition of thoracic movement. Opening of the thorax disclosed a beating heart having a frequency identical to the observed and recorded displacements.

Fig. 2 is a recording obtained from a larger rat fetus with the thorax opened and the heart exposed. In this recording distinct auricular and ventricular components are recognized. An additional feature of this recording is the presence of a partial heart block having a variable rhythm—three and four to one. During the periods of ventricular inactivity the influx of blood produces an enlargement of the heart with a corresponding shift of the surface toward the plate. This change is recorded as a slower upward deflection, upon which are superimposed the deflections due to auricular activity. The slow rise terminates with an abrupt upward deflection, which is synchronous with ventricular activity. This is followed by an abrupt fall which is concomitant with the onset of ventricular inactivity. As the result of ventricular activity there occurs the ejection of blood and a corresponding decrease in cardiac size.

*From the University of Utah Medical School, Salt Lake City.

†Due to the untimely death of Dr. Bonar, this report represents an attempt on the part of the author to call to the attention of investigators, work that was done in collaboration.

Read in part by Dr. Bonar at the Fourth Region Meeting of the American Academy of Pediatrics in Los Angeles, Calif., November 6, 1937.

Received for publication, December 16, 1938.

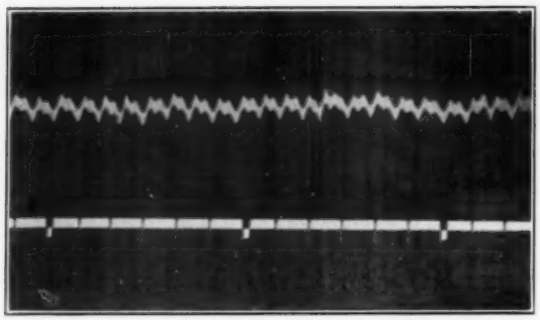

Fig. 1.

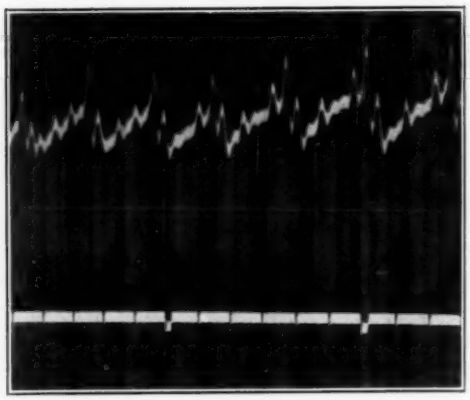

Fig. 2.

Fig. 1.—*a*, Cardiac beat. *b*, Time, one second.

Fig. 2.—1. Cardiac activity. *a*, Auricular activity. *b*, Ventricular activity. 2. Time, one second.

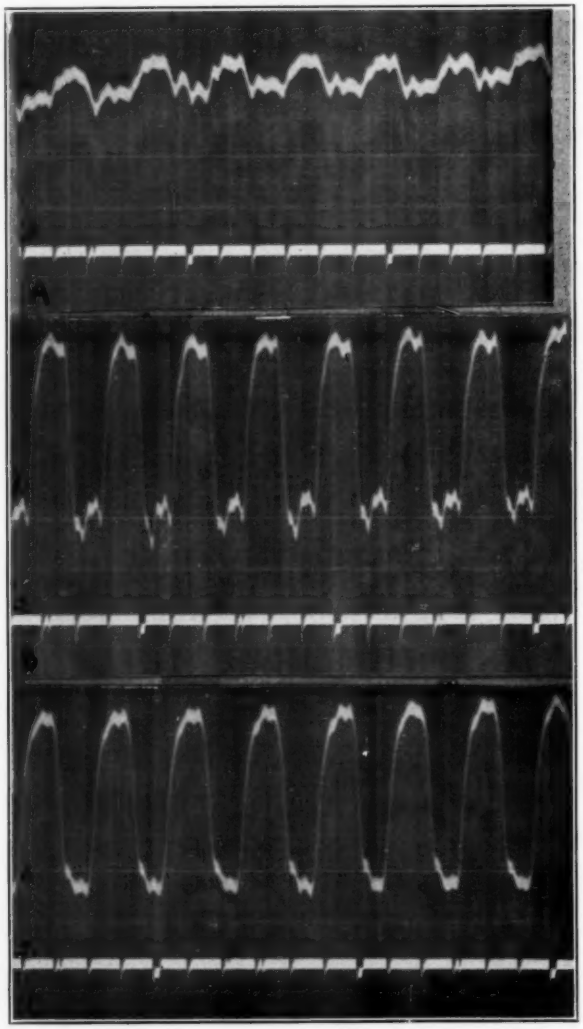

Fig. 3.—*A*. 1. Cardiac activity, plate over bulbus. 2. Time, one second. *B*. 1. Cardiac activity, plate over right auricle. 2. Time, one second. *C*. 1. Cardiac activity, plate over ventricle. 2. Time, one second.

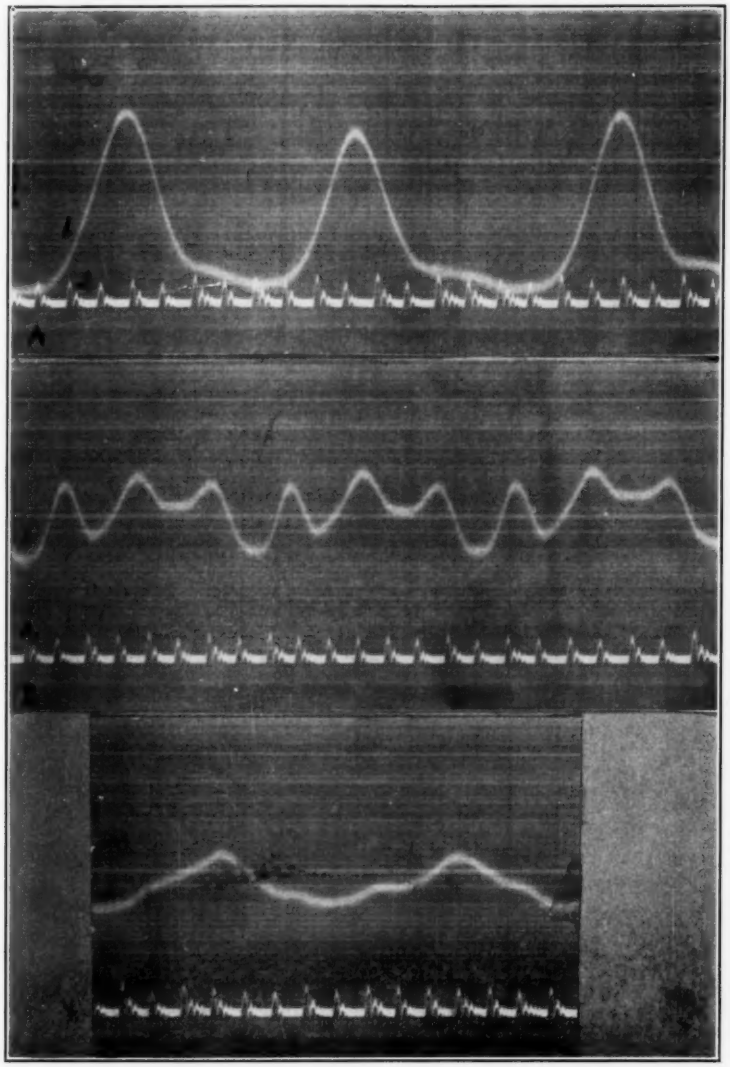

Fig. 4.—*A*, *B*, and *C* are recordings obtained from a sleeping infant, three months of age. *A*. 1. Abdominal respiration. 2. Time, one-fifth second. *B*. 1. Apex beat and thoracic respiration. 2. Time, one-fifth second. *C*. 1. Fontanel pulsations. 2. Time, one-fifth second.

Figs. 3*A*, *B*, and *C* are typical recordings obtained by placing the 3 mm. plate over various regions of the exposed three-chambered heart (turtle). With proper manipulation of the plate, any one of the displacement components may be minimized or emphasized.

Figs. 4*A*, *B*, and *C* represent curves obtained from a sleeping infant by placing the "pick up" condenser plate over the abdomen, thorax, and anterior fontanel.

Fig. 5 illustrates the appearance of curves obtained from an adult by placing the plate over the external jugular vein. The general characteristics of the fundamental venous pulse curve can be recognized. These components are marked in the conventional manner.

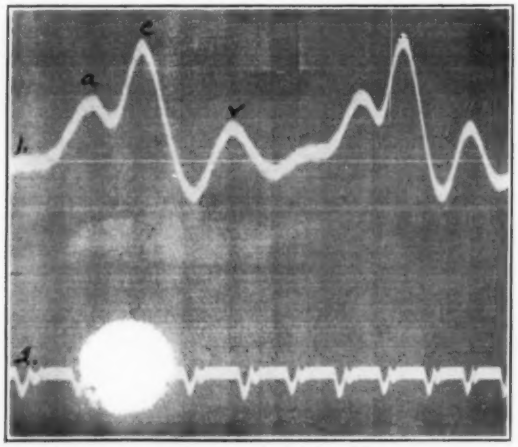

Fig. 5.—1. Venous pulse. 2. Time, one-fifth second.

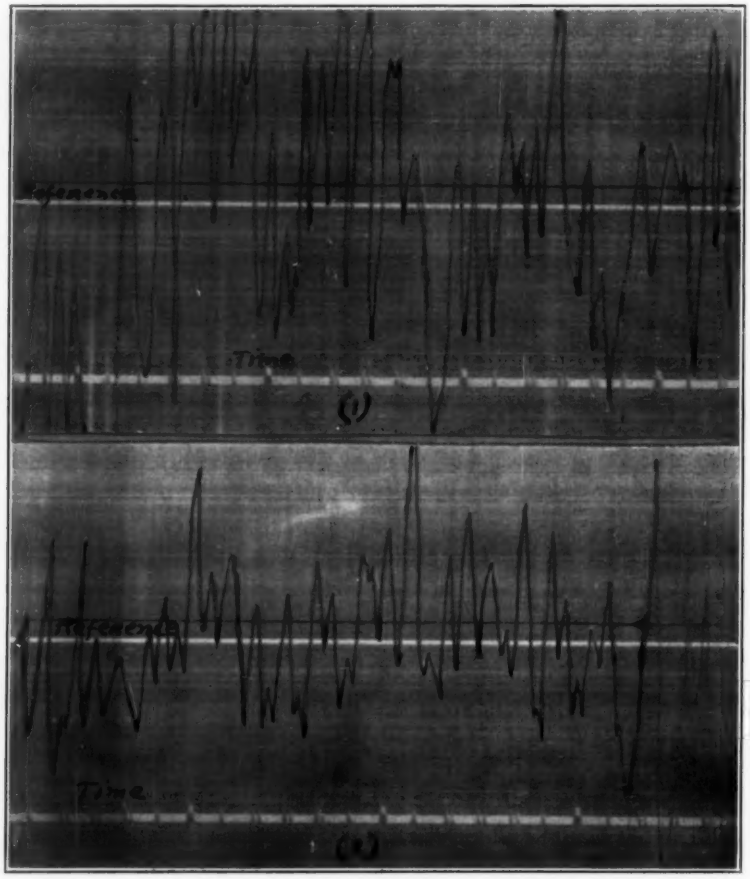

Fig. 6.—1 and 2. Time, one second.

Fig. 6 shows a possible use of the instrument for the analysis of tremors: in this case, normal hand tremors. An adult male student endeavored, with the best of his ability, to maintain the outstretched hand at a fixed distance over the plate. The subject judged the fixed distance by observing the position of a beam of light relative to a fixed point on the wall: (1) represents his ability after a number of trials, (2) represents his ability ten minutes after the ingestion of 10 c.c. of absolute ethyl alcohol diluted to 50 c.c. with fruit juice.

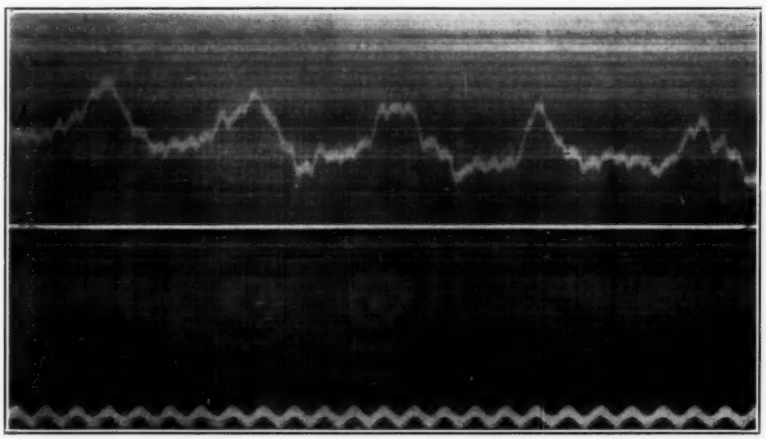

Fig. 7.—1. Kidney volume recording. 2. Time, one second.

Fig. 7 illustrates a curve indicative of spontaneous volume changes occurring in the kidney of the conscious dog. By special application an insulated plate was placed at a fixed distance from the surface of the right kidney. The animal was allowed to recover, and the recording was made seventy-two hours after the operation. Arterial pulse and respiratory and slower volume components are present.

CONCLUSIONS

The oscillato-capacigraph has been found valuable in making physiologic studies of surface changes. The instrument may be adapted to many uses. It is hoped that in the future other investigators will make use of it wherever possible so that its ultimate value may be established.

REFERENCES

1. Fenning, Con: A New Method of Recording Physiologic Activities. I. Recording Respiration in Small Animals, J. Lab. & Clin. Med. 22: 1279, 1937.
—, and Bonar, Barnet E.: A New Method of Recording Physiologic Activities. II. The Simultaneous Recording of Maternal Respiration, Intrauterine Fetal Respiration and Uterine Contractions, J. Lab. & Clin. Med. 22: 1280, 1937.
2. Bonar, Barnet E., and Fenning, Con: Studies of Fetal Respiratory Movements; Recording Methods, Am. J. Dis. Child. 55: 322, 1938.

A NOMOGRAM FOR DETERMINING THE STATISTICAL SIGNIFICANCE AND THE PROBABLE ERROR OF DIFFERENCES OF PERCENTAGES*

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THE clinician, the experimenter, and the statistician frequently resort to percentages to express their findings in regard to deaths, cures, recurrences, metastasis, incidence of disease, etc. If the percentages of two series differ, it is necessary to show that the differences are sufficiently marked to be statistically significant before one can state that the experimental series differs from the control.

The significance of differences of percentages can be determined by use of various statistical formulas. The simplest one† is

$$\chi^2 = \frac{(ad - bc)^2 \ (a + b + c + d)}{(a + b) \ (c + d) \ (a + c) \ (b + d)}$$

where *a*, *b*, *c*, and *d* represent the experimental data. The determination of χ^2 is time-consuming and laborious. It seemed that it should be possible to construct a graph to obviate the calculation.

Determination of Statistical Significance.—Fig. 1 is a nomogram for determining whether two percentages differ significantly. One may assume a hypothetical experiment to illustrate its use. Suppose it is found that the mortality rate of a group of 100 patients treated with a serum is 40 per cent, whereas in a control group of 100 patients, 53 per cent die. Can it be said from these findings that the serum has any therapeutic value? In other words, is the 13 per cent difference statistically significant?

Fig. 2A illustrates the use of the nomogram for the solution of this particular problem. First locate point *A* corresponding to 40 per cent (abscissa *p*) and 100 cases (ordinate *n*). From point *A* draw a line to the origin of the graph, *O*, and extend it to meet the *d* ordinate for the difference of the percentages (13 per cent). The point of intersection of line *OA* and the 13 per cent ordinate is at 114, according to abscissa *c*. Similarly, draw a line from the point 53 per cent (abscissa *p*) and 100 cases (ordinate *n*), through the origin *O* and intersecting the *d* ordinate for 13 per cent at point 117. Adding the two *c* determinations gives 231. If the sum is equal to or less than 200, a constant for the graph, the percentages studied may be considered statistically different.‡ In the particular case under consideration the sum 231 is

*From the Laboratory of Pathology, Veterans Administration Facility, Hines.

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†Simmons, J. S.: Laboratory Methods of the United States Army, 1935, p. 1040.

‡It will be shown later that if the sum of the *c* constants is 200, the difference of the percentages is equal to twice its standard deviation (or P = 0.046). This P value is considered significant in this paper. If a more stringent test of significance is desired, one can require that the sum of the *c* constants should be equal to or less than 128 (or P = 0.012).

slightly greater than the constant 200 and, therefore, the difference between 40 and 53 per cent is not statistically significant and has to be considered within the realm of experimental error. It may be concluded that the hypothetical serum did not have any appreciable effect on mortality rates.

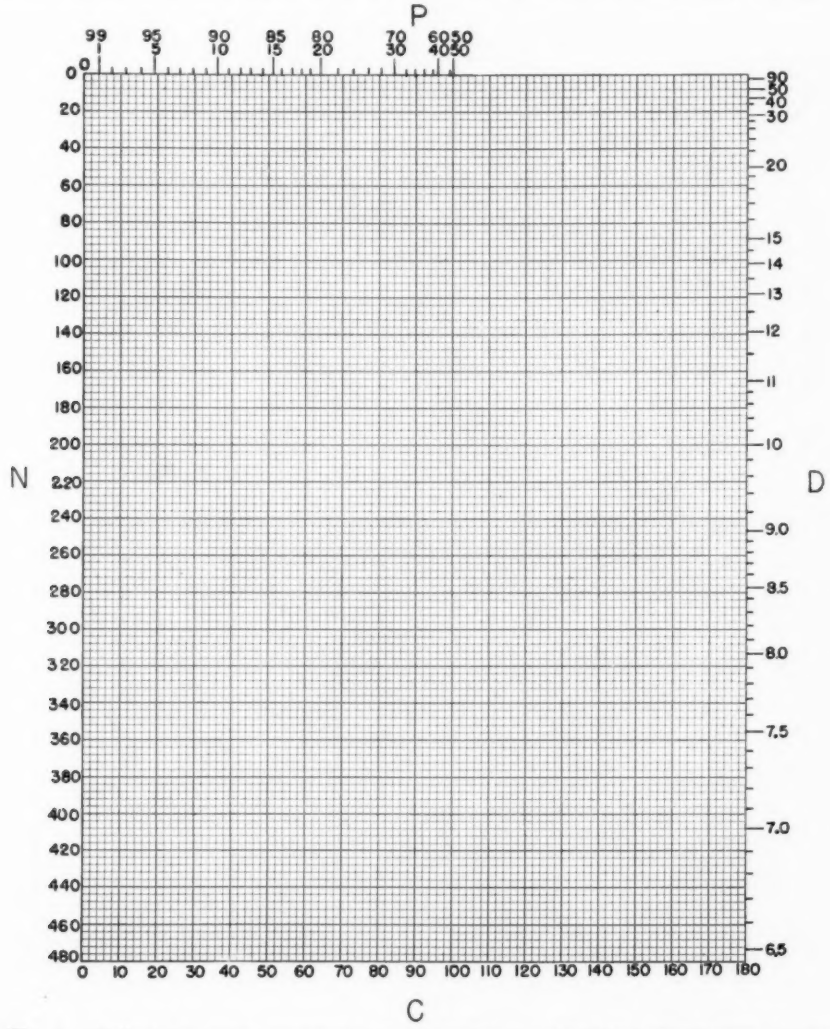

Fig. 1.—Method for using nomogram to determine the statistical significance of two percentages.

Attach a colored thread to a pin and insert pin in upper left hand corner of the graph (point O, O). With the thread taut, extend it through a point representing the number of cases in one series (using the left hand ordinate *n*) and the corresponding percentage (using either upper or lower figures of the upper abscissa *p*). Note the point at which the thread crosses the line representing the difference of the two percentages (using ordinate on the right, *d*). Read the probable constant (using the lower abscissa, *c*) corresponding to this point.

Similarly, determine a second probability constant for the second series of cases and its percentage. Add the two constants. If this sum is 200 or less, the difference of the percentages is significant.

N, number of cases; *P*, percentage; *D*, difference of percentages; *C*, probable constant.

In practice it is, of course, not necessary to draw the various lines. A thread attached to a pin inserted in point *O* can be used to determine the direction of the lines and the *c* constants.

It is surprising to note that, according to the graph, a difference between 70 per cent and 83 per cent in series of 100 each is statistically significant

(sum of c constants is 171), while the difference between 40 per cent and 53 per cent is not (sum of c constants is 231), although both pairs of series have differences of 13 per cent. Checking by statistical formulas and tables shows that P in the first instance is 0.03, which is considered significant, whereas in the second instance P is 0.07, which is not significant. The graph and the statistical formula are in agreement. Common sense could not be depended upon in these cases to tell whether the differences in the percentages are significant.

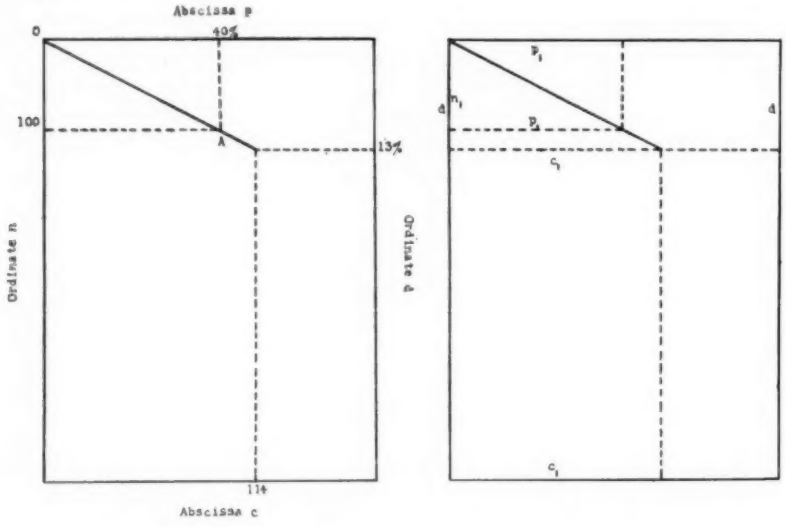

Fig. 2.—*A* and *B* show use of the nomogram. See text.

Determination of P.—For routine purposes the determination of the significance of the differences of two percentages suffices. In some cases it is desirable to determine the probable error or the value of P. These quantities can be readily determined from the sum of the c constants by use of Table I. The table shows that if the sum of c constants is 200 or less, P = 0.046 or less. It is customary to consider values having a P of 0.046 or less as statistically significant.

TABLE I

DETERMINATION OF P FROM THE SUM OF THE c CONSTANTS, AS OBTAINED FROM THE NOMOGRAM

SUM OF c CONSTANTS	VALUE OF P
50	0.000063
75	0.0011
100	0.0047
125	0.0114
150	0.0143
175	0.0325
200	0.0455
250	0.0736
300	0.1024

Proof and the Method of Construction of the Nomogram.—For those who may be interested in the construction of the nomogram, the following brief

mathematical analysis is appended. Fig. 2B represents the nomogram and the determination of the significance of the difference, D, between two percentages P_1 and P_2 based on N_1 and N_2 number of cases. The capital letters D, P_1, P_2, N_1, and N_2 represent the actual figures (as 13, 40, and 53 per cent, and 100 and 100 cases, respectively, in the specific example mentioned previously), and the small letters d, p_1, p_2, n_1, and n_2 represent the quantities in arbitrary units. If these units are determined, the nomogram can be constructed.

Considering the similar triangles in Fig. 2B, it is seen that

$$(1)\quad \frac{p_1}{n_1} = \frac{e_1}{d}$$

Similarly, it can be shown that

$$\frac{p_2}{n_2} = \frac{e_2}{d}$$

then, by addition

$$(2)\quad \frac{p_1}{n_1} + \frac{p_2}{n_2} = \frac{e_1 + e_2}{d} = \frac{e}{d}$$

Let u, v, and w be arbitrary constants of construction and let

$$p_1 = u\,P_1\,(100 - P_1)$$
$$p_2 = u\,P_2\,(100 - P_2)$$
$$n_1 = v\,N_1$$
$$n_2 = v\,N_2$$
$$d = \frac{w\,v}{u\,D^2}$$

Substituting in (2)

$$(3)\quad \frac{u}{v}\left(\frac{P_1\,(100 - P_1)}{N_1}\right) + \frac{u}{v}\left(\frac{P_2\,(100 - P_2)}{N_2}\right) = e\,\frac{uD^2}{wv}$$

Solving for D

$$(4)\quad D = \sqrt{\frac{w}{e}}\sqrt{\frac{P_1\,(100 - P_1)}{N_1} + \frac{P_2\,(100 - P_2)}{N_2}}$$

According to statistics

$$(5)\quad S = \sqrt{\frac{P\,(100 - P)}{N}} \qquad \text{where}$$

S is the standard deviation of percentage P

Then

$$(6)\quad S_1^2 = \frac{P_1\,(100 - P_1)}{N_1} \text{ and } S_2^2 = \frac{P_2\,(100 - P_2)}{N_2}$$

Therefore

$$(7)\quad D = \sqrt{\frac{w}{e}}\,\sqrt{S_1^2 + S_2^2}$$

According to statistics

(8) $S_D = \sqrt{S_1^2 + S_2^2}$ where S_D is the standard deviation of the difference D. Dividing (7) by (8)

$$(9)\quad \frac{D}{S_D} = \sqrt{\frac{w}{e}} \quad \text{and} \quad e = w\left(\frac{S_D}{D}\right)^2$$

Assume that difference D is significant if D = 2 times its standard deviation, i.e.,

$$\text{if } \frac{S_D}{D} = \frac{1}{2} \text{ or } c = w\left(\frac{1}{2}\right)^2 = \frac{w}{4}$$

It is to be concluded, then, that two percentages, P_1 and P_2, differ significantly if the nomogram shows that c is equal to or is less than $\frac{w}{4}$, a constant (Q.E.D.).

For construction of the nomogram these formulas suffice:

$$p = u\ P\ (100 - P)$$

$$n = v\ N$$

$$d = \frac{w\ v}{u\ D^2}$$

Fig. 1 was constructed on 2 mm. graph paper with
u = 0.04 mm., v = 0.5 mm., and w = 800 mm.

SUMMARY

Percentages or rates cannot be said to differ appreciably until they are shown to have significant differences.

The statistical significance of differences of percentages can be calculated by formulas, but this method is time-consuming and laborious.

By means of the nomogram it is possible to determine whether two percentages have significant differences. The nomogram can also be used to find the P value of the difference of two percentages.

A SIMPLIFIED APPARATUS FOR THE PRESERVATION OF BACTERIAL CULTURES IN THE DRIED STATE*

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CINCINNATI, OHIO

DURING an intensive bacteriologic study of the stools of infants and young children with acute enteritis it became desirable to have at our disposal a simple apparatus for the preservation of some of the bacteria in a viable state without the loss of virulence, immunologic or biochemical characteristics. There are numerous reports in the literature regarding the length of time bacteria survive without change after being dried from the frozen state. Swift[1] reported that hemolytic streptococci dried from the frozen state in 1916 to 1917 were still viable and possessed their group specific substance in 1937. Lancefield,[2] in her studies with hemolytic streptococci preserved by freezing and drying over a period of twelve years, observed that such cultures consistently maintained the characteristics noted at the time of their original

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preservation. Pneumococci similarly preserved maintained their type specificity. Rake[3] observed that meningococci maintained their original virulence and specific carbohydrate content for at least twenty months when preserved in a similar manner.

The general principle involved in these reports is that of removing water by distillation under reduced pressure from material while it is frozen. Two general methods are employed. In the one, material is previously frozen in a dry ice bath (solid carbon dioxide), attached quickly to a suction pump, and the water vapor condensed in a dry ice condenser.[4] In the other method, which has been developed by Flosdorf and Mudd,[5, 6] the material is first "degassed" under somewhat reduced pressure and then subjected to very low pressure while the water vapor is absorbed by a chemical desiccant. Flosdorf and Mudd use drierite (anhydrous calcium sulfate).

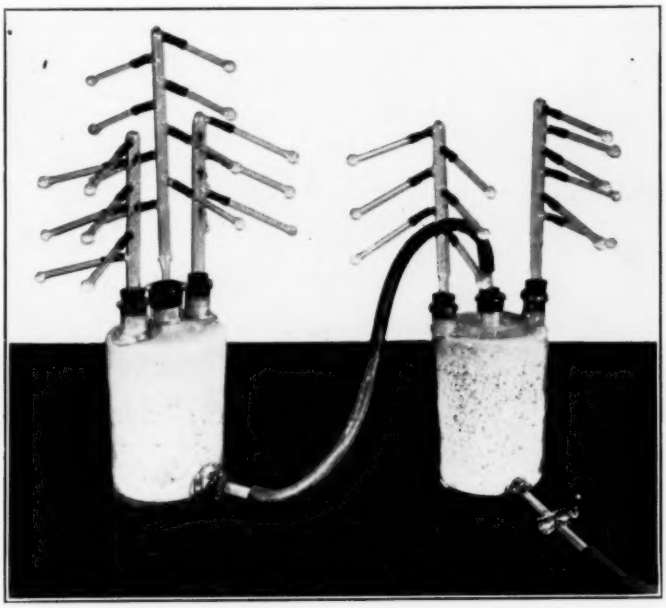

Fig. 1.—Apparatus for dehydrating small quantities of bacterial cultures from the frozen state.

We have utilized the latter method, i.e., the freezing and removal of water vapor under reduced pressure and its absorption on a chemical desiccant, in devising a simple apparatus from material available in most laboratories. The availability of the apparatus, its simplicity of construction and operation, and the economy in time, together with the satisfactory results obtained, prompt us to describe it.

APPARATUS

The equipment consists of a vacuum pump similar to a Hyvac pump, capable of reaching a pressure of approximately 0.2 mm. Hg; a good grade of pressure tubing; pyrex Woulff bottles of 2 liter capacity; 9 mm. pyrex tubing from which bulb containers may be easily blown; a cross-fire oxygen-gas torch for sealing the bulb containers under vacuum; a suitable desiccant,

such as drierite; incidentals, such as rubber stoppers, rubber connections, and large bore pyrex glass tubing from which the manifolds can be made. This apparatus is illustrated in Fig. 1. A spark coil vacuum tester is of much assistance in locating any very slight leak in the apparatus. It is our custom to go over the entire apparatus as soon as a good vacuum has been established, and in this way locate and eliminate any tiny leaks which, if permitted to remain, would result in slow and improper desiccation.

The bulb containers are blown from pieces of 9 mm. pyrex tubing, 8 cm. long, so that the bulb capacity is 0.5 to 0.8 c.c., facilitating drying 0.2 to 0.3 c.c. of culture. The manifold is made from 15 mm. pyrex tubing to which are attached 6 or 8 side arms of 6 mm. pyrex tubing, 2 cm. long, set at an acute angle of 70°. We dry 32 ampoules of cultures daily, using five such manifolds.

METHOD AND PROCEDURE

The method is essentially that of vacuum drying from the frozen state without preliminary freezing in dry ice or an ice salt bath. This method has been very well described by Flosdorf and Mudd[6] and for further details the reader is referred to their study. Briefly, our routine procedure is as follows: Pieces of rubber tubing, 2 cm. long, are attached to each of the side arms on the manifolds. The manifolds are then wrapped separately in paper and sterilized in the autoclave for twenty minutes at 15 pounds pressure. The Woulff bottles have previously been filled with regenerated drierite and stoppered tightly. At the time of drying, a sterile manifold is fitted into one of the openings in the Woulff bottle-desiccating chamber with a good grade of rubber stopper, preferably of gum. An eighteen-hour-old salt-free broth infusion culture of the microorganism to be preserved is mixed with an equal volume of sterile skim milk and 0.2 or 0.3 c.c. of the above mixture is added, without contamination, to the previously plugged and sterilized bulbs. Milk seems to prevent excessive bubbling during the degassing period and facilitates rapid resuspension of the dried bacteria in water whenever desired at a later date. The insertion of the manifolds in the Woulff bottles before connecting the bulbs simplifies manipulation and decreases the possibility of contamination. These bulbs are attached to the rubber connections on the manifold with the aid of an ordinary rubber tube stretcher, the width of the jaws being reduced so that touching the rubber connections by hand is unnecessary. Occasional flaming of the exposed ends of the rubber tube connections and the tongs reduces the danger of contamination. After loading all manifolds the rubber stopper connections between the manifolds and the Woulff bottles are greased, tightly inserted, and the vacuum pump started. The system is allowed to evacuate, and the stopcock between the pump and Woulff bottle is closed simultaneously with the development of the first bubble. The cultures are then allowed to degas for approximately ten minutes, at the end of which time evacuation is continued. The cultures will freeze and the containers frost over after about three to five minutes of pumping, if the degassing process is complete and the system is free from leaks. The degassing period should be extended in case bubbling continues as the cultures are being

pumped to freeze. After vacuum freezing the moisture vaporizes to the drierite with sufficient rapidity to retain the cultures in the frozen state throughout the remainder of the desiccating period which is approximately four hours.

The tubes are left under vacuum for a total of eighteen to twenty hours to insure complete drying. At the end of this period the ampoules are cut off near the rubber connection under vacuum by means of the cross-fire oxygen-gas torch. The cultures are thus sealed under vacuum in all-glass containers and are stored in the refrigerator until subcultured. According to the literature, the above conditions are most ideal for preservation of bacteria. Upon subculturing, the ampoules are marked with a file near the bulb and opened in a flame with the aid of asbestos gloves. During this procedure care should be taken to avoid heating the organisms. After opening the container, the subculture medium is introduced with a sterile, slender-tipped pipette. The desiccated materials are exceedingly hygroscopic, and there is no difficulty in obtaining a suspension of the organisms. We have had no difficulty with contamination while using the above procedure.

The drying capacity of the drierite is limited, but it may be readily regenerated by heating in an oven overnight at a temperature ranging from 160 to 200° C. A shorter regeneration period, at higher temperature, is recommended by the distributors of drierite, but for routine convenience the former conditions are found to be more satisfactory. The drying capacity of the contents of each Woulff flask may be estimated and regeneration carried out when necessary since 20 gm. of anhydrous drierite is adequate desiccant to freeze and dry 1 c.c. of culture suspension. The rubber connections are renewed at intervals, depending upon the nature of the tubing and the frequency of their use.

We have used this apparatus extensively during the past summer and have found it satisfactory for drying and preserving cultures. From August 10 to August 31 we made 12 runs and dried and preserved 354 vials of cultures. We started with 5 pounds of freshly regenerated drierite and used it throughout the 12 runs without regeneration. We find upon subculturing these dried cultures six months later that they are viable and have retained their original cultural characteristics. This study is in progress and further observations on these dried cultures are planned.

We have also found this apparatus satisfactory for concentrating and drying human blood serum from the frozen state by the Flosdorf and Mudd technique.

SUMMARY

A simple, inexpensive, low vacuum apparatus is described which satisfactorily freezes and dries 32 bacterial cultures daily. The small amount of time required for operation is much in its favor. The simplicity of the apparatus and procedure makes low temperature desiccation of heat labile substances available to the average laboratory.

We are indebted to Mr. Frederic E. Holmes, of the Children's Hospital Research Foundation, for blowing the glass manifolds.

REFERENCES

1. Swift, H. F.: Simple Method for Preserving Bacterial Cultures by Freezing and Drying, J. Bact. **33**: 411, 1937.
2. Lancefield, R. C.: Serological Differentiation of Human and Other Groups of Hemolytic Streptococci, J. Exper. Med. **57**: 571, 1933.
3. Rake, G.: Viability and Virulence of Frozen and Dried Cultures of Meningococcus, Proc. Soc. Exper. Biol. & Med. **32**: 975, 1935.
4. Elser, W. J., Thomas, R. A., and Steffen, G. I.: Desiccation of Sera and Other Biological Products (Including Microorganisms) in Frozen State With Preservation of Original Qualities of Products So Treated, J. Immunol. **28**: 433, 1935.
5. Flosdorf, E. W., and Mudd, S.: Procedure and Apparatus for Preservation in "Lyophile" Form of Serum and Other Biological Substances, J. Immunol. **29**: 389, 1935.
6. Flosdorf, E. W., and Mudd, S.: Improved Procedure and Apparatus for Preservation of Sera, Microörganisms and Other Substances—Cryochem-process, J. Immunol. **34**: 469, 1938.

AN AMPLIFIER FOR RECORDING HEART SOUNDS THROUGH THE USE OF THE CATHODE-RAY TUBE*

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MANY workers who are interested in the recording and study of heart sounds make use of battery-operated amplifiers and some form of galvanometer-recording device.[1-5] In considering the several types of instruments in use, the conclusion may be reached that these systems have shortcomings worthy of mention. For example, batteries require replacement and constant care; they require a separate carrying case or a large single case to house both the amplifier and batteries; the voltage of the several types of batteries used is subject to continuous change, and as a result the amplifying characteristics of the amplifier are variable. Further, battery-operated tubes tend to be microphonic due to their structural characteristics. As for string or mirror galvanometers, they must be critically standardized to avoid overshooting; they may have inherent resonant periods and harmonic distortions which frequently fall directly in the range of the sound frequencies under investigation. It would appear that the medical profession has assumed the string galvanometer a suitable recording instrument, but electrical engineers do not wholly agree with this contention. Thus, Reid[6] indicates as two major defects of the string galvanometer: (1) Above 20 cycles per second distortion in the form of harmonics, takes place, and as a result the string does not respond equally well to high and low frequencies. (2) The presence of body resistances of about 2,500 ohms in the string circuit will cause the string to react in a manner not easily controlled or compensated for.

Recognition of the importance of these factors, coincident with a study of electronic devices for more accurately recording heart sounds, has led to

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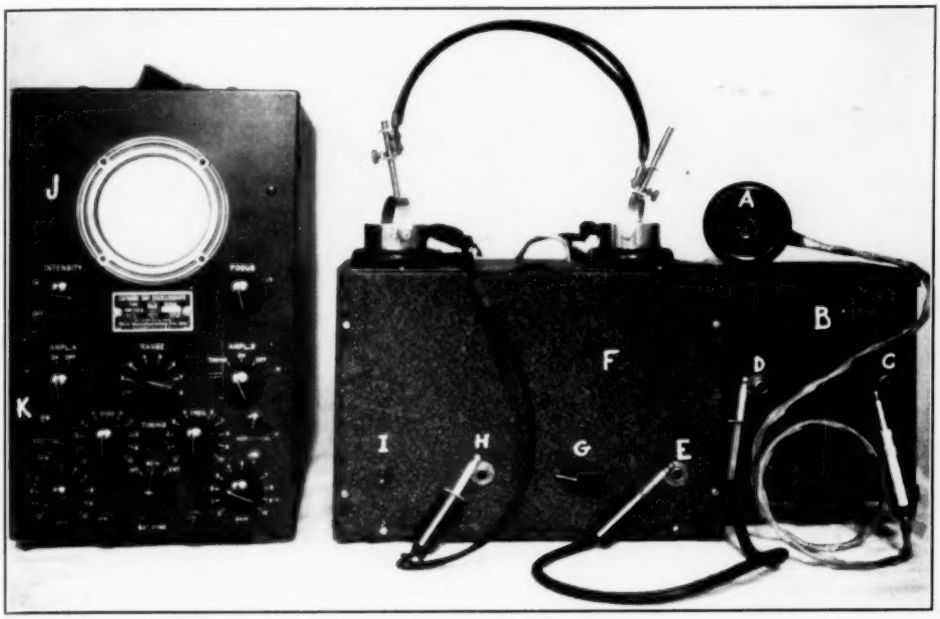

Fig. 1.—The cathode-ray stethograph, less camera. *A*, crystal microphone; *B*, preamplifier; *C*, input; *D*, preamplifier output to *E* or *K*; *E*, amplifier input; *F*, main amplifier; *G*, volume control; *H*, headphone output; *I*, line switch; *J*, viewing screen; *K*, oscillograph input.

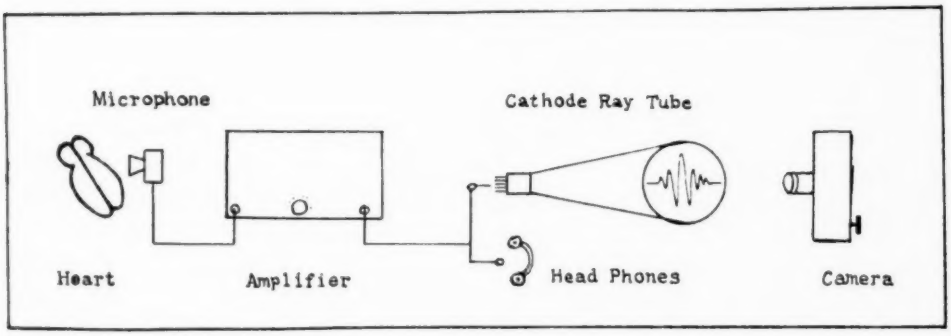

Fig. 2.—Pictorial representation of apparatus used.

the development of an A.C. operated amplifier which can be used either as an electrical stethoscope, or, in conjunction with a cathode-ray tube, as a cathode-ray stethoscope. A photograph of the apparatus which has been developed is shown in Fig. 1. The sketch given in Fig. 2 presents the arrangement of the several components of the instruments used in the heart sound studies. The microphone used is of the piezoelectric type, that is, one containing a magnesium sulfate crystal which produces minute voltages when sound waves impinge upon its surface. The resulting voltage varies in accordance with the amplitude and frequency of the sound wave and is impressed on the grid of the first tube of the amplifier. This type of microphone is small, light, and sturdy, and may be used in any position. Its frequency response is excellent; it possesses no hiss or background noise, and it requires no matching transformers or batteries.

The amplifier has been designed to have high gain for frequencies as low as 30 cycles per second and to dampen frequencies higher than 1,000 cycles per second. This provision in the design of the instrument has been included since heart sounds usually have a frequency range of from approximately 40 to 200 cycles. Thus, with the unnecessary higher frequencies damped out, a major cause of interference is eliminated. The tubes used are of the metal type. The power supply is taken from any common wall source of 110 volts, A.C. The power transformer is placed within the main cabinet which also includes the rectifier tube, an efficient filter circuit, and two amplifying tubes. Inspection of the photograph presented in Fig. 1 shows a preamplifier, a small metal box attached on the right of the larger box. This unit consists of a two tube circuit of particularly high gain and sensitivity,

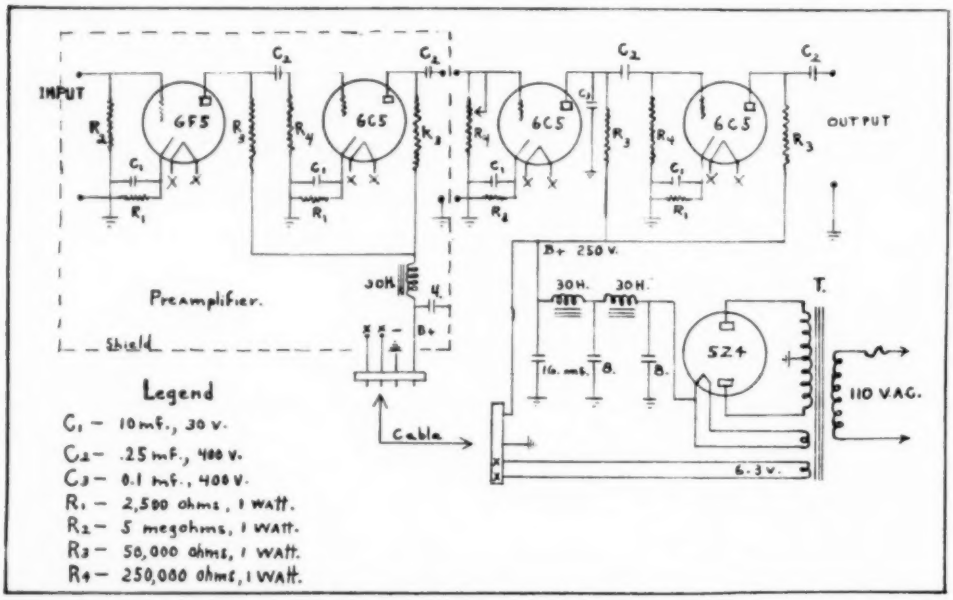

Fig. 3.—Schematic diagram of amplifier.

a characteristic necessary for adequate amplification of the low output of the crystal microphone before it is introduced into the main amplifier. On the front of cabinets B and F in Fig. 1 may be seen four holes to receive the four plug-in jacks. From right to left the holes are microphone input, preamplifier output, main amplifier input, and headphone output. The connecting cable with jacks is used between the two units when all tubes are desired in the circuit. The preamplifier is the condenser output and may be used to actuate the galvanometer on an electrocardiograph or the cathode-ray oscillograph seen in Fig. 1.

The schematic diagram of the entire amplifier is given in Fig. 3. It may be noted that the preamplifier is shielded from the chassis containing the power transformer, and that the B-plus supply has additional filtering within the preamplifier. These two conditions contribute greatly to making the amplifier quiet and hum free.

With the headphones plugged into the preamplifier alone, the volume of the heart sounds is about equal to that of the ordinary stethoscope. With the entire amplifier in use, the volume control needs to be advanced less than half its extent to obtain more than sufficient volume for the headphones. For the purpose of recording, the output of the preamplifier is impressed directly on the input of the cathode-ray oscillograph. If a string or mirror galvanometer is employed, the preamplifier output is introduced directly into the string circuit.

The cathode-ray tube is not a new device, since, in fact, it preceded the development of the ordinary radio tube.[7] It may be traced back to the famous Geissler tube from which was developed the Crookes tube. In 1897 Dr. F. Braun, a German physicist, modified the Crookes tube to make the first cathode-ray tube. He did this by placing a metal shield with a hole in it in the path of the electrons passing toward the target or screen. Consequently, instead of a cloud of electrons proceeding to the target end of the tube, only a pencil of electrons passes through the hole in the shield. In this way, a method of focusing and varying the size of electron beam was attained. Further experimentation demonstrated that this beam, when striking certain substances, was capable of causing them to fluoresce. Previously, it had been discovered that a beam of electrons could be bent to one side or the other by electrical or magnetic influence. Braun and his co-workers combined his innovation with the previous discoveries to produce the modern cathode-ray tube.

The cathode-ray tube thus has three essentials: the pencil of electrons, a fluorescent target for this pencil to write on, and some method of bending the beam in response to whatever changes it is desired to measure. These essentials of the tube have not been changed since Braun's time, but many significant improvements have been made in details, such as making the electron pencil sharper and more responsive, improving the fluorescent screens, lowering the operating voltages, and lengthening the tube's life.

In referring to Fig. 2 it may be seen that the modern tube is flask-shaped, the electrons are produced in the narrow neck of the tube by a heated filament, and the beam strikes the base or target and traces visibly the phenomena being studied. The photograph in Fig. 1 shows a modern cathode-ray unit with the various controls for focus, intensity, frequency, and amplitude of the beam.

The tube has many applications[8, 9] and is used throughout the industrial world, in television, in aviation for blind flying, and, more recently, in the medical world. In connection with the last application, it is used in several physiology laboratories for the study of Berger's wave of the brain, muscle and nerve responses, registration of the heart sounds, and in electrocardiography. Concerning this last use, Frey[10] has stated that the cathode-ray oscillograph is superior to moving coil oscillographs, chiefly because it will register accurately at all vibration frequencies present in carrying out a study of the heart.

Experience with the visual registration of heart sounds on the screen of the tube reveals that the wave forms may be observed instantly, accurately,

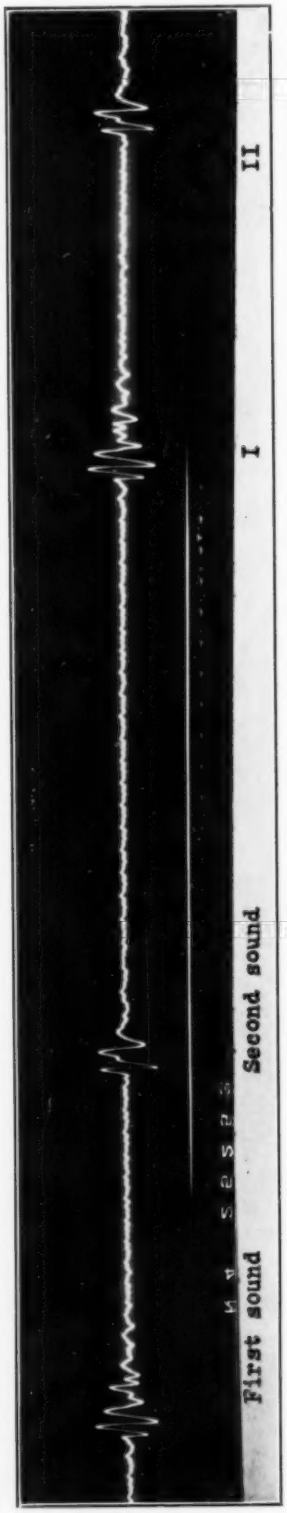

Fig. 4.—Heart sound tracing from cathode-ray tube. Film speed 200 mm. per second.

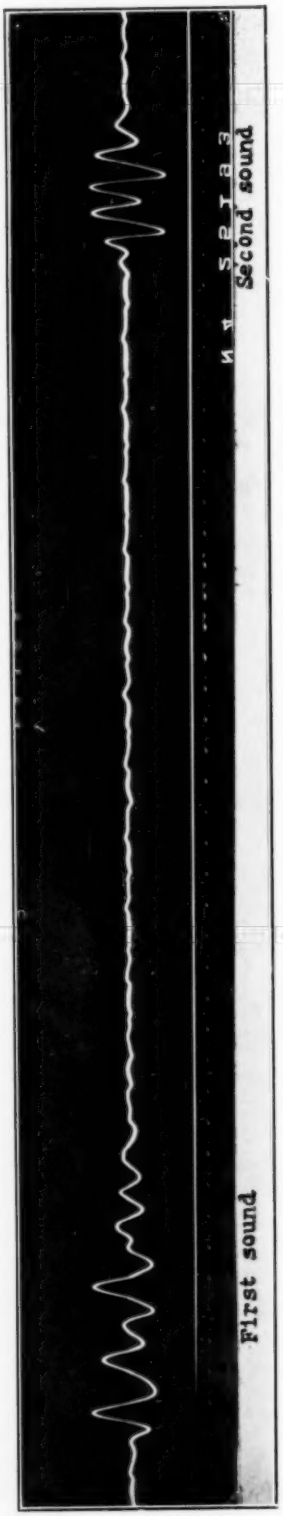

Fig. 5.—Tracing similar to above but with film speed of 400 mm. per second.

and with any desired amplitude. Although disturbances of rhythm, rate, and sounds can be seen on the screen, they are in essence transitory and pass rapidly. In this respect, visual observations of heart sounds, without provision for permanent recording, assume the same shortcomings which are inherent in the use of the stethoscope. For this reason graphic recording is essential for detailed study of the sound events in the cardiac cycle. As shown in Fig. 2, a recording camera using a moving film was necessary to photograph the movement of the electronic pencil on the target.

The sample tracings of heart sounds shown in Figs. 4 and 5 were photographed from the screen of a blue spot, short persistence cathode-ray tube using a running film camera having an f/2.5 lens. The film used was 35 mm. supersensitive panchromatic.

In Fig. 4 a heart sound tracing is shown illustrating two cardiac cycles. The film speed was approximately 200 mm. per second. The base line can be seen to have present a faint 60 cycle ripple, the cause of which was the lack of adequate shielding of the tube at the time the tracing was taken. A slight halo accompanies the recording spot, this being due to the glow of adjacent particles of the fluorescent material of the tube screen. Fig. 5 illustrates a single heart sound cycle from the same patient but with a film speed advanced to 400 mm. per second. At this speed the spot halo is not as noticeable. The speed of 200 or 400 mm. per second is far greater than is necessary for routine stethograph studies, but has been reproduced here to demonstrate the practicability of this method of recording. The optimum speed for heart sound recording is probably 100 mm. per second.

SUMMARY

A description is given of a heart sound amplifier which uses a cathode-ray tube as a recording device for reproducing in the form of photographic tracings the sound patterns emanating from the functioning heart.

The author is indebted to W. C. Mock, Jr., of the U. S. Bureau of Standards, for assistance in producing the cathode-ray tracings in Figs. 4 and 5.

REFERENCES

1. Bierring, W. L., Bone, H. C., and Lockhart, M. L.: Use of Electrostethograph for Recording Heart Sounds. Preliminary Report, J. A. M. A. **104**: 628, 1935.
2. Schwarzschild, M. M., and Feltenstein, M. D.: A New Method for the Recording of Heart Sounds, Am. Heart J. **10**: 453, 1935.
3. Johnston, F. D.: The Value of Sound Records in the Diagnosis of Mitral Stenosis, Am. Heart J. **10**: 654, 1935.
4. Sacks, H. A., Marquis, H., and Blumenthal, B.: A Modification of the Wiggers-Dean Method of Recording Heart Sounds Using Audio Amplification, Am. Heart J. **10**: 965, 1935.
5. Waud, R. A.: An Improved Electropolygraph, J. Lab. & Clin. Med. **21**: 864, 1936.
6. Reid, W. D.: Preliminary Report on Use of Improved Form of Electrocardiograph, J. Lab. & Clin. Med. **17**: 804, 1932.
7. Free, E. E.: Writing Sound's Autograph, Radio News **12**: 1056, 1931.
8. Washburne, R. D.: The Versatile Cathode-Ray Tube, Radio-Craft **7**: 599, 628, 1936.
9. Rider, J. F.: The Cathode-Ray Tube at Work, New York, 1935, J. F. Rider.
10. Frey, H.: The Efficiency of Various Electrocardiographs, Ztschr. f. Kreislaufforsch. **29**: 41, 1937; Am. Heart J. **13**: 750, 1937 (Abst.).

THE EAGLE COMPLEMENT FIXATION TEST FOR SYPHILIS

A Note on the Amboceptor Titration

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WITH the increasing use of the complement fixation test for syphilis, as outlined by Eagle,[1] it seems likely that some will encounter certain difficulties met in our laboratories at the time of our first experiments with the technique in October, 1937. For that reason, it may be well to detail the major difficulty, and the means by which it has been satisfactorily overcome.

In our hands, the test, run in parallel with an eighteen-hour fixation and both Kahn and Eagle flocculation tests, proved at first definitely inferior in sensitivity. At the same time, complement titrations set up with the Eagle fixation test indicated the presence in the reaction tubes of considerably more than the 2½ units of complement required. In reviewing the theoretical considerations of the test,[2] it was found that in an effort to secure 2½ units of complement in the test proper, use is made of the supposed approximately reciprocal relationship between amboceptor and complement, and the amboceptor is titrated daily, using 2½ units of amboceptor, thus determined, to sensitize the sheep erythrocytes for the test. It was apparent from the data that, either because of unusually potent complement or some similar factor, the concentrations used were outside the range in which the relationships between amboceptor and complement are even approximately reciprocal.

However, it seemed advantageous to retain, if possible, the principle of daily amboceptor titrations, since as Eagle has pointed out, and as data previously secured had confirmed, the day-to-day variation in resistance of sheep erythrocytes to hemolysis, as measured by titration with amboceptor, is much greater than the day-to-day variation in the complementary activity of fresh guinea pig serum secured from a fairly large pool of carefully selected animals. It appears logical to assume that the daily titration with amboceptor may be retained, and control of complement concentration simultaneously achieved, if the amboceptor titration be set up with complement two-fifths the concentration of that employed in the test, and the unit of amboceptor so secured be used to sensitize the sheep erythrocytes. The test proper must then of necessity contain 2½ units of complement per tube.

In practice, the tests are first set up, precisely as outlined by Eagle[1] with complement diluted 1:10, after which a portion of the residual complement is further diluted to give a final dilution of 1:25 (1.4 ml. of 1:10 complement added to 2.1 ml. of isotonic saline suffices for seven tubes), and the 1:25 dilution is used in the amboceptor titration, which in all other respects is carried out in accordance with the standard technique. The highest dilution of amboceptor which gives complete hemolysis in thirty minutes is then used to sensitize the sheep cells (and not, let it be pointed out, 2½ times this amount, as with the original method).

With this minor modification, the Eagle complement fixation test for syphilis has, in a series of more than 10,000 cases, given a high degree of sensitivity, with a specificity rating certainly in excess of the requirements of the United States Public Health Service.

REFERENCES

1. Eagle, H.: Modifications in the Eagle Antigens for Use in the Complement Fixation and Flocculation Tests for Syphilis, and Minor Changes in the Technic of These Tests, J. Lab. & Clin. Med. **22**: 300, 1936.
2. Eagle, H.: The Laboratory Diagnosis of Syphilis, St. Louis, 1937, The C. V. Mosby Co., p. 153.

ILLUMINATING BOX FOR FLOCCULATION (KAHN) AND SEDIMENTATION TESTS

James S. Brotherhood, M.D., Grand Rapids, Mich.

THE satisfactory performance of any flocculation or sedimentation tests depends upon the proper illumination of the test tubes. Reliance upon daylight, especially during the winter months, and upon ordinary electric light, is not always satisfactory.

The construction of an illuminating box, which will permit the passage of light through the sides of the test tubes, as well as from below through the depth of the fluid, helps to solve this difficulty. In order to have the light pass from beneath, it is necessary that the lower cross support of the test tube rack be replaced by a piece of clear glass, and the rack be painted with a dull black paint to prevent reflected light.

The box shown in Fig. 1 is made of galvanized sheet iron, the size of the box depending upon the width of the test tube rack. A convenient size which will accommodate the ordinary test tube rack is 13 inches wide, 10 inches high, and 7 inches deep. A small recessed platform is made to extend across the front of the box, and it is upon this platform that the test tube rack is placed.

The base and back of this platform are made of two panels of daylight blue flashed opal transilluminating glass which may be obtained from any x-ray supply house.

The interior of the box is painted with a flat white paint; the exterior with a flat black paint. On the posterior wall of the box, at the top and bottom are two rows of half inch holes to permit ventilation and for the passage of electric wire from the lamps to the socket.

For illumination two 60 watt frosted electric light bulbs are placed, one at either end of the upper portion of the box, so that the light is reflected downward, and through the two panels of glass. The top of the box is fitted with a cover to permit replacement of the bulbs when burned out (Fig. 2).

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Fig. 1.

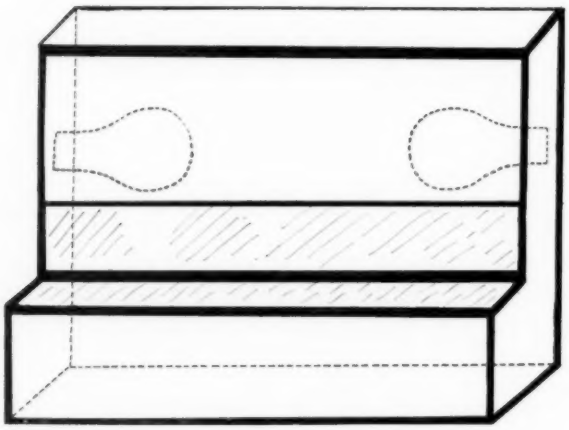

Fig. 2.

It is found of advantage to have a narrow strip of black paper glued to the bottom of the upright panel of glass to permit a break in the area of light passing through glazed area. The box should be made light enough to permit easy handling, so that when the test tube is in position on the platform, the whole apparatus may be lifted to level of the eye and viewed.

The cost of making the box by a tinsmith, including paint, electric bulbs, sockets, wiring and other incidentals was less than five dollars.

329 Metz Building

THE SEALING OF MUSEUM JARS*

M. H. Book, M.D., and M. W. Sullivan, M.D., Boston, Mass.

TWO devices which have been of value in the sealing of flat-top museum jars are described here. They are particularly useful to those who do not have enough of this work to acquire the proficiency of experts.

The cement used depends on several considerations, but long experience has shown that the best preparation is one composed principally of asphalt. Formulas are given by Little[1] and Mallory.[2] We have been using a battery sealing compound† with satisfactory results. Most other cements are open to the objection that they are dissolved by one or more of the reagents used in the preparation of the specimen.

The gas-heated cement applicator shown in Fig. 1 enables one to apply a neat ribbon of the softened cement to the edge of the jar in a short time. The container for the cement is made of two pieces of tin, soldered together with high melting point solder in the shape of a scoop about 6 cm. long. The open end is pinched together with pliers to leave an opening 1 mm. wide. The back of the scoop is screwed to the end of a wooden handle, such as is used in chisels. The bottom of the handle is grooved to take the gas line, bent in the shape shown. A blowpipe makes a satisfactory gas line. Staples are used to fasten it to the handle, but it should be left just loose enough so that its position with respect to the scoop may be altered if necessary.

A good method of affixing the top in order to obtain a perfect seal is to heat it when it is in the proper position and apply pressure evenly. An ordinary household electric iron is useful for this purpose, but for large jars it is desirable to have a heater with a larger surface. The heater shown in Fig. 2 was made for this purpose. Into a cake tin 7 by 11 by 1¼ inches is poured 5 pounds of refractory cement,‡ mixed with water. In this is embedded a 660 watt replacement heating coil, which may be obtained from any hardware store. The ends are attached by bolts to a length of asbestos-covered electric wire. A wooden handle fastened by wires to the ends of the pan completes the device.

The actual procedure in sealing a jar is as follows: The mounted specimen is carefully placed in the jar, and the preserving fluid is added to within 2 cm. of the top edge. The latter, as well as the lid, should be scrupulously clean and dry. With the applicator a ribbon of the melted cement is deposited on the edge of the jar, an attempt being made to keep to the outer

*From the Department of Anatomy, Tufts College Medical School, Boston.
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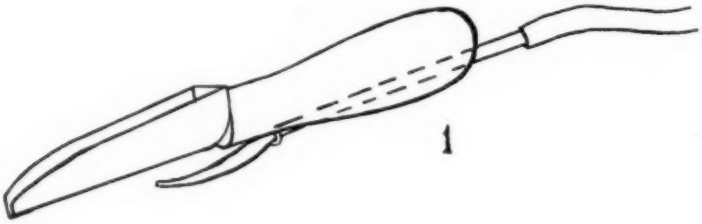
†RP Battery Sealing Compound No. 359, manufactured by the H. H. Robertson Co., Pittsburgh, Pa.

‡Alundum refractory cement RA 162, obtainable from the Central Scientific Co., Cambridge, Mass.

surface. The lid (which must have a small hole in the back left-hand corner) is placed in position, and the electric iron or heater, at room temperature, on top. The current is then turned on, and as the lid warms up it softens the cement. When a broad seal all the way around is obtained, the heater is removed. Care should be exercised not to have the seal too thin, otherwise it may be easily broken by jarring or temperature changes. When the cement



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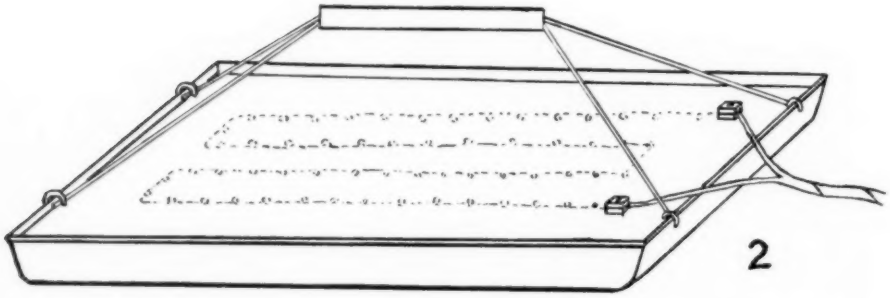

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is thoroughly cooled, preservative fluid is added through the vent with a needle and syringe to 1 cm. from the top. It is not wise to have the air cushion over the fluid too thin, as a period of hot weather might break the seal of the vent. The latter is closed with a drop of hot cement. Excess cement is scraped off, and the jar is cleaned with gasoline. The seal may be made more attractive by applying a narrow border of black enamel, using masking tape to obtain a straight edge.

No matter how carefully the jar has been sealed, it should be examined from time to time, as temperature changes occasionally cause a break in the seal, especially at the vent.

REFERENCES

1. Little, J. L.: The Museum in the Department of Pathology of the University of Toronto, Its Arrangement and Utilization for Teaching Purposes, Equipment and Technique, J. Tech. Methods **13**: 15, 1934.
2. Mallory, F. B.: Pathological Technique, Philadelphia, 1938, W. B. Saunders Co., p. 384.

AN INSTRUMENT FOR OBTAINING BONE MARROW*†

G. O. Favorite, M.D., Philadelphia, Pa.

THE trephine and the needle puncture are the current popular methods for obtaining bone marrow for study. The sternum is the usual source of the marrow, and the present methods are more or less designed for this bone. The needle biopsy is easily performed, but the material obtained usually consists of a mixture of blood and marrow cells. Quite often the lack of cellularity in the preparation does not represent the true state of the marrow. On the other hand, the trephine method often requires the services of the surgeon. As a diagnostic procedure it must be admitted that it is formidable, and, at times, out of proportion to the information obtained. The disadvantages are outweighed by the cellularity of the preparations and material obtained for histologic sections in studying the topography of the marrow. An excellent critique of the comparative methods is given by Dameshek, Henstell, and Valentine.[1] They conclude, in part, that the trephine method is far superior to the puncture technique.

It seemed that there should be a compromise between these two extremes, that is, ease of performance with poor cellularity of the needle method, and comparative difficulty of performance with good cellularity and topography of marrow with the trephine. With this thought in mind, an instrument has been devised which seems to strike a happy medium. In no way is it intended to replace the trephine. On the other hand, it has been found far superior to the needle method since actual marrow is obtained for smears, and in many instances, for histologic sections.

The instrument consists of a No. 11 gauge cannula, a trocar, and a small drill which fits the cannula, as shown in Fig. 1. The cannula and trocar have been used for some years by our laboratory in bleeding donors. The drill is fitted with locking nuts which regulate the extension of the drill beyond the cannula. The tip has shallow cross grooves which aid in retaining the marrow. In the adult of average skeletal development, this distance is 4 mm.

The gladiolus is the site of operation. The most desirable location is the level of the third or fourth intercostal space. This region is especially desirable in women for cosmetic reasons as well as for the cellularity of the marrow.

The technique of operation is easily acquired after several performances. After sterilization of the surface with alcohol, the skin, soft tissues, and periosteum are infiltrated with a local anesthesic, such as 1 per cent novocain. The periosteum is infiltrated for an area of 1 cm. in diameter. If the infiltration is adequate, the patient experiences no pain. The cannula, with the trocar in place, is introduced obliquely through the skin, in the direction of the long axis of the sternum. Since the caliber of the cannula is rather large,

*From the Clinical Laboratories, Hahnemann Hospital, Philadelphia.
Received for publication, March 11, 1939.
†The instrument is manufactured by the Scientific Equipment Co., Philadelphia.

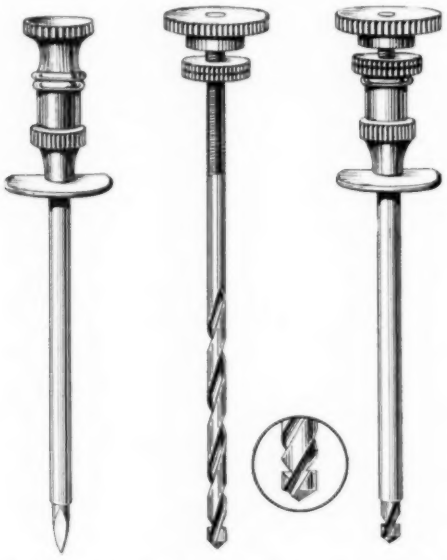

Fig. 1.—From left to right, the figure shows the cannula and trocar, the drill with the details of the tip in the circle, and the drill within the cannula.

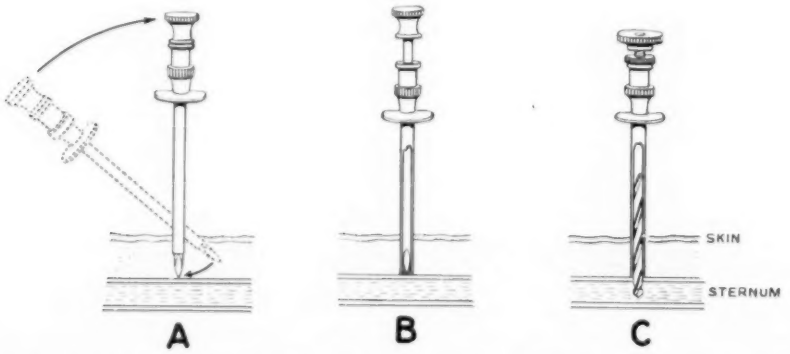

Fig. 2.—*A*. The dotted figure shows the direction for introducing the cannula and trocar through the skin. When this maneuver is completed, the instrument is rotated in the upright position. *B*. The cannula is pushed downward to rest on the sternum. *C*. The drill has penetrated the sternal plate and the tip is within the marrow cavity.

the skin must be fixed firmly against the chest wall with one hand while the cannula is penetrating the skin. If this is not done, the skin will buckle, and considerable force is then required to penetrate it. It is essential that the cannula itself, regardless of the trocar, must enter the skin for a distance of at least 0.5 cm. The cannula and trocar are then straightened in the upright position, as shown in Fig. 2A. The trocar should touch the periosteum in the center of the anesthetized area. With the trocar as a guide, the cannula is forced downward so as to rest on the periosteum. This maneuver is illustrated in Fig. 2B.

The trocar is removed, and the drill is introduced in the cannula, which now serves as a guide and as a guard. It also determines the depth to which the drill will enter the sternum. One must ascertain previously that the locking nuts are firmly tightened at the proper distance. The drill is operated by

firmly turning and applying a varying amount of force downward until it catches. Thereafter it will proceed downward by merely rotating it. When the drill has reached its full predetermined depth (Fig. 2C), it is further rotated several times, and then withdrawn while the cannula is held firmly in place. The marrow will be found in the grooves of the drill. With the point of a needle (gauge 19) the fragments can be removed and smears made on clean slides by gently streaking the marrow on the glass surface. The bevel of the needle will hold the fragments in position for this purpose.

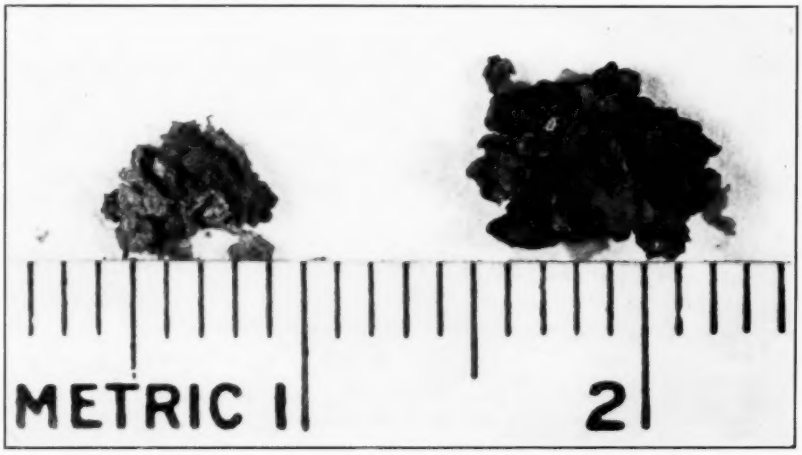

Fig. 3.—A photograph showing the amount of marrow material obtained in two cases.

The drill should be reintroduced several times. It is essential that the cannula be held firmly against the sternum in the upright position so that the original hole is not lost. By varying slightly the direction of the cannula and drill, additional marrow is obtained for histologic sections. Fig. 3 represents the average amount of material obtained in two of the 50 cases in which the instrument has been used.

The cannula is withdrawn in the usual manner. There is left a small puncture wound of the skin. This can be minimized by pressing it between the fingers, thus flattening and approximating the edges. It is then covered with a dry sterile dressing.

In conclusion, we feel that this instrument, essentially a drill, is a worthy compromise between a needle and a trephine. It offers all the advantages of the needle and most of those of the trephine. The cellularity of the smears is similar to the trephine preparations. Small marrow fragments often give a fair topography of the marrow but do not compare with the large sections obtained with the trephine. The method can be performed at the bedside and can be repeated many times.

REFERENCE

1. Dameshek, W., Henstell, H. H., and Valentine, E. H.: The Comparative Value and the Limitations of the Trephine and Puncture Methods for Biopsy of the Sternal Bone Marrow, Ann. Int. Med. **11**: 801, 1937.

230 N. Broad Street

ADAPTATION OF THE ROLLS RAZOR TO A NEW TYPE OF MICROTOME BLADE*

J. M. Feder, M.D., Anderson, S. C.

THERE exists a palpable need for an auxiliary microtome blade in the larger laboratories, an all-purpose blade in small laboratories and an inexpensive one to be used by students in their first attempt at microtomy.

The Rolls blade, held in a suitable holder, meets most of the requirements of each group. When so employed, it can be used in connection with all types of microtomes, sliding, rotary, or "swing." It works equally well with paraffin or frozen sections, and as a consequence, can be instantly changed from one type of service to another.

The orthodox microtome blade is a dangerous weapon in the hands of beginners, and the blade described greatly minimizes the risk of personal injury by the inexperienced worker.

For the past two years an attempt has been made by us to evaluate all of the various blades now in use in an effort to select one that would most nearly meet the major requirements for a universal edge. The Rolls razor was selected for the following reasons:

1. The Rolls blade has approximately the same degree of hardness as the finest microtome blade and is made from the very best English steel.

2. The blade nearly approaches in shape the ordinary microtome knife. (Rolls razors are hollow ground; however, through the cooperation of the makers, blades purchased for this purpose will be "chisel ground" if requested. When sharpened in this manner, they are much more satisfactory.)

3. The unique construction of this razor makes honing and stropping easy for the most inexperienced in this otherwise difficult procedure. One lid of the box contains a hone, while the other contains a fine strop. The blade is attached to a sliding mechanism by a half turn, and the stropping handle moved backward and forward. The edge is brought into contact with strop and hone at the proper angle and is automatically reversed when the maximum excursion has been reached.

This ease of stropping and honing assures a keen-edged knife at all times, and the facility with which the blades can be stored aids in keeping them in a safe place when not in use.

*From the Laboratories of Anderson County Hospital, Anderson.
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Holder for Rolls Blades.—A piece of tool steel, 12.5 cm. in length and 0.5 cm. in thickness, was tapered down toward a blunt edge, approximately as nearly as possible the shape and size of an ordinary microtome blade. The holder measured 2.9 cm. in breadth, and at the thinnest portion measured 0.2 cm.

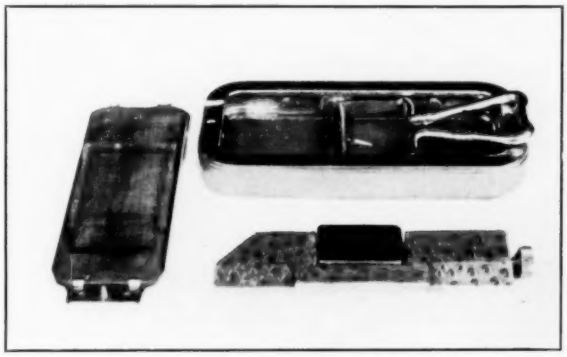

Fig. 1.—Holder with blade in position. *Above*, holder, stropping and honing appliance is shown. Hone is in position for use, and strop is shown at left. By the expedient of placing strop on box and removing that part containing hone, the situation is reversed, and stropping can be carried out.

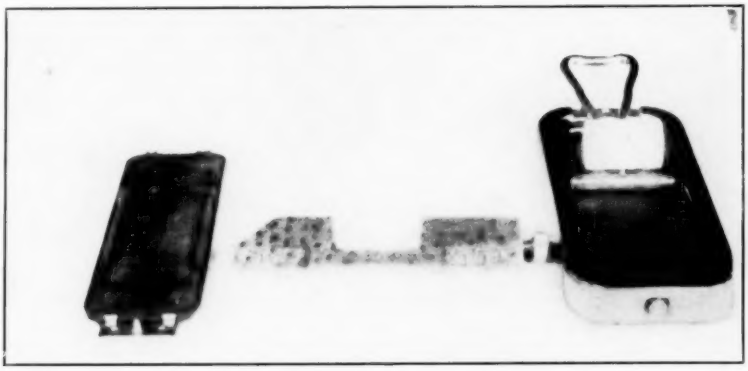

Fig. 2.—Holder with blade detached. The blade is in sharpening element ready for honing. It can be attached and removed by a simple half turn.

In the central portion of this bar of steel, a slot was cut to accommodate the blade. This opening is 3.8 cm. long and 1.5 cm. deep. On the left end of this opening, two pins were placed to engage in the two depressions noted on one end of a Rolls razor. The opposite end of this blade is fitted with an inset steel ball. A setscrew with a knurled head is placed in the right end of the holder and is so arranged, that when it is tightened, its inner end engages the ball of the blade, thus securing it in an immovable position in the holder.

To Insert Blade.—The blade is placed in the depression of holder in such a manner that the two small pins on the left engage in proper depressions in blade. The setscrew is tightened, and the instrument is ready for use. Insertion and removal of blades are operations requiring but a few seconds to accomplish.

CONCLUSIONS

A microtome blade suitable for all light routine work has been described. It has been found to function as well as any of the commonly used appliances and is superior from the standpoint of safety and the ease with which sharpening can be accomplished.

It is economical to purchase and maintain, and it offers no storage problem when not in use.

Appreciation is extended at this time to Mr. Raymond C. Corby, of Rolls Razors, Inc., for his aid and counsel during this investigation.

The apparatus described here is distributed by A. S. Aloe Co., St. Louis, Mo.

A SIMPLE METHOD OF STAINING SPIROCHETES IN ROUTINE PARAFFIN SECTIONS*

With Remarks Regarding Distribution of Spirochetes in Tissues

Gabriel Steiner, M.D., Detroit, Mich.

A METHOD of staining spirochetes in frozen sections was published in this Journal December, 1937.[1] A modification of this technique for use with paraffin sections in routine procedure was sought, and a reliable method has been developed.

The procedure is as follows: the tissue is thoroughly fixed in 10 per cent formalin. Fresh tissue can be fixed in hot formalin (the formalin solution being changed once).

1. The tissue is dehydrated in graded alcohols, cleared in xylol, embedded in paraffin, cut at 9 to 10 micra, and mounted with albumin.

2. The mounted sections are deparaffinized in xylol and placed in absolute alcohol (two changes).

3. The sections are placed in a uranium nitrate-alcoholic gum mastic solution for one to one and one-half minutes. This time should not be exceeded, since a longer exposure to uranium nitrate interferes seriously with the subsequent staining of the spirochetes.

Uranium nitrate-alcoholic gum mastic solution:

Uranium nitrate (4%), alcoholic (absolute)	20 c.c.
Gum mastic (25%), alcoholic (absolute)	40 to 50 c.c.
Absolute alcohol	20 to 30 c.c.

This gives an 0.8 per cent uranium nitrate 10 to 12.5 per cent gum mastic solution in absolute alcohol. Two hundred cubic centimeters of the solution are necessary when using a glass tray carrying ten slides.

*From the Department of Pathology, Wayne University College of Medicine, Detroit.
Received for publication, April 6, 1939.

4. Wash the slides in at least three changes of distilled water. The first washing will cloud the water. A few streaks of the gum mastic may be shed in the second washing. If the third change of water is not clear, a fourth should be employed.

5. Place the slides in a 0.1 per cent aqueous silver nitrate solution for one to one and one-half hours. The staining dish containing the slides and silver nitrate is placed in a water bath at 100° C.

The solution of silver nitrate must be chemically pure. We have found the rectangular staining dish with the removable glass tray most convenient. A pan of tap water over an electric plate makes a satisfactory water bath. After the staining dish has been placed in the pan of warm tap water, the temperature is gradually raised to boiling.

6. Wash the slides in distilled water.

7. Dehydrate in ascending graded alcohols—80 per cent, 95 per cent, 100 per cent.

8. Place sections in 10 to 12.5 per cent alcoholic (absolute)-gum mastic solution for five minutes.

9. Repeat step 4.

10. Reduce in 5 per cent hydroquinone solution for twenty to thirty minutes. The staining dish containing the slides and hydroquinone solution is placed in a water bath at 100° C., the temperature having been raised gradually.

Hydroquinone solution:

Hydroquinone	10 gm.
Alcoholic gum mastic (12.5%)	1 c.c. (approximately)
Distilled water	200 c.c.

11. Wash thoroughly in distilled water, dehydrate in alcohol, clear in xylol, and mount in balsam.

12. If desired, the tissue may be counterstained with hematoxylin and eosin, cresyl violet, acid fuchsin, basic fuchsin, etc., after washing following reduction with the hydroquinone.

Preparation of Stock Solutions:

1. Uranium nitrate	4 gm.
Alcohol (100%)	100 c.c.

This solution may be kept indefinitely in an amber, glass-stoppered bottle.

2. Silver nitrate (C. P.)	1 gm.
Water (distilled)	1,000 c.c.

This is a stable solution if kept in an amber, glass-stoppered bottle.

3. Gum mastic powder	25 gm.
Alcohol (100%)	100 c.c.

Shake several times over a period of two days and then filter through a double filter. The filtrate is clear and has a yellow color.

The hydroquinone solution is unstable and must be prepared just prior to using.

The uranium nitrate-alcoholic gum mastic solution may be used not more than twice.

Separate staining dishes should be used for the uranium nitrate-gum mastic, for the aqueous silver nitrate, and for the hydroquinone solutions. Individual staining dishes should also be used for the distilled water and alcohol.

RESULTS

The color of the stained tissue depends upon the thickness of the section. Sections 8 to 9 micra in thickness have a deep yellow color; thicker sections tend to be brown, while thinner sections are pale yellow.

Occasionally reticular (argyrophil) fibers are stained in these preparations. However, because of their grayish color, as contrasted to the jet black of the spirochetes, reticular fibers are easily identified.

The time required for staining, if no counterstain is employed, is from two to two and one-half hours. Paraffin sections of primary and secondary syphilitic skin lesions, gummata, brain in general paresis, syphilitic aorta, the lung, liver, intestine and kidneys of congenital syphilitics, and organs of guinea pigs with Leptospira infection have been stained. The contrast between the glistening metallic black surface of the spirochetes and the indifferent yellow tissue is very marked. This marked contrast permits the identification of spirochetes when present in the tissue even in very small numbers.

Advantages of This Method.—1. Precipitates of metallic silver are prevented more easily than in other methods.

2. The method is faster than any other.

3. Only four special chemicals are required: (a) uranium nitrate, (b) silver nitrate, (c) gum mastic, and (d) hydroquinone.

Silver nitrate in 0.1 per cent solution is used; therefore, only small amounts of this rather expensive material are required.

4. This method fits into the usual routine technique used for paraffin sections in this country; therefore, alternating serial sections can be stained with hematoxylin eosin and for spirochetes. Counterstains are readily made on sections treated for presentation of spirochetes.

Special Arrangements and Distribution of Spirochetes in Tissues.—In organs of the *congenital syphilitic* with abundant spirochetes, an arrangement along the connective tissue bundles is very often found. The spirochetes show a tresslike accumulation on the connective tissue strands and between them. The longitudinal axis of the spirochetes runs parallel to the longitudinal direction of the fiber strands. In the outer parts of the walls of cross-sectioned blood vessels, dense masses of spirochetes are found arranged circularly. In epithelial structures (bronchial epithelium, intestinal epithelium) the spirochetes tend to be arranged radially to the lumen, parallel with the longitudinal axis of the columnar cells, and mostly between the cells. The basal membranes show a different arrangement insofar as here a circular distribution of spirochetes is seen. A clear-cut transverse section of a bronchus or an intestinal villus shows that the angle between the direction of the spirochetes in the epithelium and that of the organisms beneath the epithelium is usually

90 degrees (Fig. 1). In the muscularis of the intestinal tract the arrangement of spirochetes is in a line running parallel to the direction of the muscle fibers. Thus, in the layer of circularly arranged muscle fibers the spirochetes are arranged circularly also, and in the longitudinal muscular layer a longitudinal location of the spirochetes is found. In the leptomeninges the same rule applies—the spirochetes are arranged along the longitudinal course of the connective tissue, tangentially to the surface of the cortex of the brain.

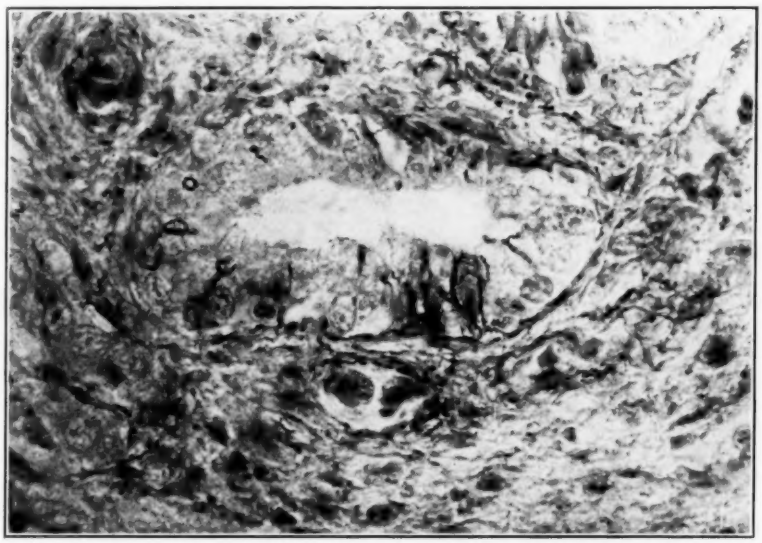

Fig. 1.—Routine paraffin section, new stain, congenital syphilis. Spirochetes are arranged radially to the lumen of the duct parallel with the longitudinal axis of the columnar cells. In the basal membrane and the adjacent connective tissue spirochetes show a circular distribution. Note degenerating forms of spirochetes, rings, and knobs in parts of the duct. (×1,000.)

In paretic brains four different arrangements of spirochetes are discernible: first, a longitudinal arrangement in the adventitia of the veins, capillaries, and arteries along the course of these vessels running toward the depth of the gray matter (Fig. 2); second, an almost complete limitation to the gray substance of the central nervous system; third, an arrangement on the border line between the cortical gray matter and the white matter where the massed spirochetes tend to parallel the arrangement of the arcuate or U fibers;[2] and fourth, an arrangement around the larger nerve cells of the cortex, sometimes in dense accumulations[3] without penetrating into the nerve cells themselves. In paretic brains the complete absence of spirochetes in the vascular lumina is interesting to note, since in congenital syphilis the vascular spaces contain spirochetes. Previous examinations of a series of paretic brains with the method of frozen sections showed that, particularly in very acute and untreated cases, the parasites migrate from the leptomeninges into the cerebral cortex by way of the pial funnels of the vessels and the adventitial part of the vascular walls. A direct penetration from the leptomeninges through the superficial glial membrane and other structures of the outer part of the molecular layer was not seen. This finding is confirmed by the new method (Fig. 3). In the cerebellum of a paretic brain the same type of in-

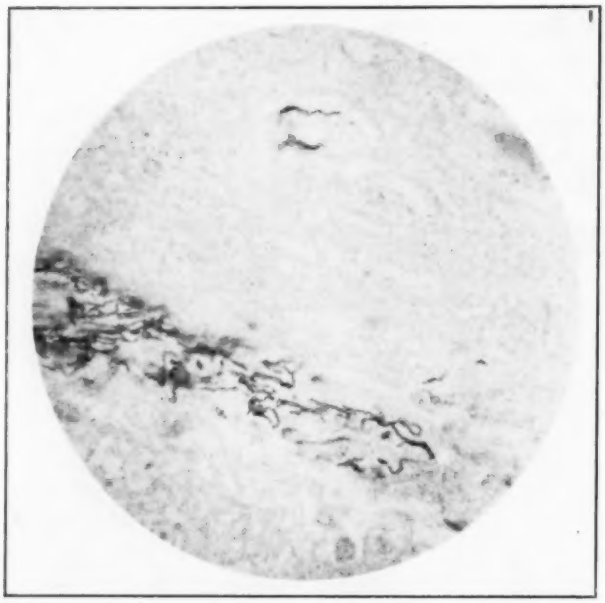

Fig. 2.—Routine paraffin section, new stain. Early acute nontreated case of general paresis. Brain cortex. Longitudinal arrangement of spirochetes in the adventitial walls of cortical blood vessels. The spirochetes in the upper part of the figure are located in the wall of a capillary. (×950.)

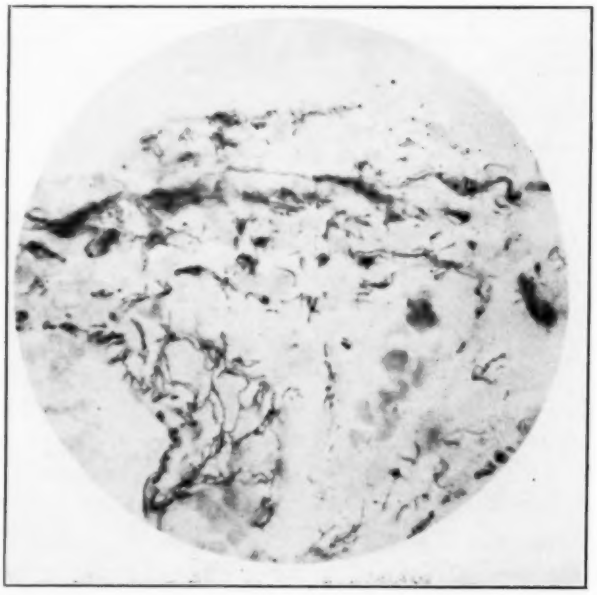

Fig. 3.—Routine paraffin section, new stain. Early acute nontreated case of general paresis. Pia-arachnoid and pial funnel entering brain cortex. In the left lower part of the figure a semicircularly shaped region of the brain surface, free from spirochetes, is seen. (×950.)

vasion was found. The spirochetes in the cerebellar substance itself are seen in parallel-running courses, and in the molecular layer sometimes they are arranged perpendicularly to the course of the blood vessels entering from the meninges.

A peculiar appearance of spirochetes should be mentioned, that is, their arrangement in large spherical or oval-shaped masses of closely packed individuals. This is seen in the acute and early stages of general paresis. These massive spirochetal accumulations are similar to those found in the liver and other organs of cases of congenital syphilis. Focal necroses in the tissues are produced by these dense masses, and this destruction is called "miliary necrosis," to be distinguished from miliary gummatous lesions. The histologic equivalent of these spirochetal massive coils is a miliary necrosis in the paretic brain also. These miliary necroses were first seen by Nissl[4] long before the disclosure of *T. pallidum* in the brain of paretics by Noguchi.[5] Later on, Hauptmann,[6] Schob[7] and others, could show that a definite and direct relationship exists between these massive spirochetal accumulations and the necrotic foci.

These massive balls of spirochetes in the acute and early stage of general paresis are found in the first, the second, and up to the fifth layer of the cerebral cortex, and on the borderline between gray and white matter. They are rarely found bordering the surface of the cerebral cortex adjoining the pia mater, but when this does occur, the shape of the spirochetal mass is semispherical or semioval, with the base on the cortical surface. On examination of the adjacent leptomeninges numerous spirochetes are also found, but the arrangement of these is in bundles of parallel-running spirochetes forming twisted threads.

One characteristic feature of the parenchymatously located massive spirochetal balls may be noted. The center shows a brownish color, whereas the spirochetes of the outer or peripheral parts are as black as usual.

Degenerating forms of spirochetes, spirochetal rings, disks and knobs of regular or irregular shape are stained black also, and are seen mostly on one end of the individual spirochete (Fig. 1). Occasionally a ring or knob is seen in the middle of a parasite.

COMMENT

The method herein described for staining spirochetes in paraffin sections is at least equal in its effects to other commonly used methods of staining spirochetes. The Levaditi,[8] Noguchi,[5] and Jahnel[9] methods are silver salt reduction methods which can be used only on tissue blocks. The Warthin-Starry[10] and Dieterle[11] methods may be useful for paraffin sections, but they are more complicated or take longer than this method.

The peculiar arrangements of *T. pallidum* in the tissues of syphilities are not at present satisfactorily explained. Some of the difficulties of explanation depend on the fact that the spirochetes may survive the death of the host. After death of the infected host, the spirochetes may move and change their position. In animals (experimental syphilis of rabbits) after surgical removal

of syphilitic tissues and immediate fixation in formalin, spirochetal accumulation along the strands of connective tissue, and parallel-running with them can be seen. Similarly the adventitial and periadventitial accumulation of spirochetes is shown. Therefore, the characteristic distribution of spirochetes in the adventitial parts of the vascular walls must be accepted as an intravital phenomenon occurring in the body of the host.

The restricted location of the spirochetes which are limited to the gray matter and absent from the white matter of the brain in general paresis is in complete accord with the clinical and histologic findings. In general paresis inflammatory reactions are seen especially in the cortical gray matter and in the gray matter of the basal ganglia (nucleus caudatus, claustrum, putamen, and thalamus opticus). They consist of an infiltration of the adventitial sheaths of the vascular walls by plasma cells and lymphocytes. These are the same spaces in which in early stages of general paresis the spirochetes are carried.

SUMMARY

1. A new simple method of staining spirochetes in routine paraffin sections and its results are described.

2. A reason for the adventitial and perivascular round-cell infiltration seen in syphilitic tissue is suggested.

REFERENCES

1. Steiner, G.: Simple Method for Demonstration of Spirochetes in Frozen Sections, J. LAB. & CLIN. MED. **23:** 315, 1937.
2. Steiner, G.: Krankheitserreger und Gewebsbefund bei multipler Sklerose, Berlin, 1931, J. Springer, p. 40 and Fig. 3.
3. Steiner, G.: Nissl's Paralysestudien und der heutige Stand der Metasyphilislehre, Arch. f. Psychiat. **87:** 126, 1929.
4. Nissl, F.: Quoted by Steiner, Ibid., p. 148.
5. Noguchi, H., and Moore, I. W.: A Demonstration of Treponema Pallidum in the Brain in Cases of General Paresis, J. Exper. Med. **17:** 232, 1913.
6. Hauptmann, A.: Ueber herdartige Spirochaetenverteilung in der Hirnrinde bei Paralyse, Monatschr. f. Psychiat. u. Neurol. **45:** 59, 165, 1919.
7. Schob, F.: Ueber miliare Nekrosen und Abscesse in der Hirnrinde eines Paralytikers und ihre Beziehungen zur Spirochaeta pallida, Ztschr. f. d. ges. Neurol. u. Psychiat. **95:** 588, 1925.
8. Levaditi, C.: A propos de l'impregnation au nitrate d'argent des spirochetes sur coupes, Compt. rend. Soc. de biol. **60:** 134, 1906.
9. Jahnel, F.: Handb. d. Haut- u. Geschlechtskr. von J. Jadassohn, **17,** Part I. Berlin, 1929, J. Springer.
10. Warthin, A. S., and Starry, A. C.: Staining of Spirochetes in Cover-Glass Smears by Silver-Agar Method, J. Infect. Dis. **30:** 592, 1922.
11. Dieterle, R. R.: Method for Demonstration of Spirochaeta pallida in Single Microscopic Sections, Arch. Neurol. & Psychiat. **18:** 73, 1927.

THE CONSTRUCTION OF AN INEXPENSIVE POWER WASHER AND THE DESIGNING OF ECONOMICAL AND EFFICIENT BRUSHES FOR THE CLEANING OF LABORATORY GLASSWARE*

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THE problem of cleaning glassware for bacteriologic and serologic work becomes momentous if more than a few workers or students are to be supplied. Often there is not a great abundance of glassware, so the soiled glassware has to be cleaned and returned for use as rapidly as possible. Likewise the space provided for cleaning purposes usually does not provide accommodation for a staff of cleaners, nor do budgets make provisions for such a staff.

Since much of the glassware in bacteriologic laboratories is contaminated with pathogenic microorganisms, the material has to be sterilized before it can be handled by the workers. The sterilization is usually done by the quickest, most efficient and most economical method—autoclaving. Often the glassware contains blood, serum, Loeffler's medium, or such media as are used for growing the acid-fast group of microorganisms. After materials of this nature have been autoclaved, their removal from the glassware is usually very difficult. Often the autoclaving process makes traces of other material more difficult to remove. In order to clean such soiled glassware it must be thoroughly brushed, and, when cleaned by hand, this procedure requires much time and energy. Under these conditions it was our experience that the glassware was not satisfactorily cleaned. Mechanical washers available on the market were either too costly or impractical for the usual teaching and research laboratory, so we set about to construct an inexpensive power washer. We were handicapped by the lack of satisfactory brushes even when washing the glassware by hand.

Power Washer.—The articles used for constructing an inexpensive and practical power washer are:

1 General Electric motor, model 5KH43AB198, 1/6 HP, 110 V, AC, 60 cycle, single phase, cushion brace
1 3/4 inch chuck, tapered
1 shaft 3/4 inch diameter, 14 inches long, tapered to fit above chuck
2 3/4 inch flat boxes with grease cups
2 thrush bearings
2 collars for above thrush bearings
1 3 inch driver step pulley to fit 3/4 inch shaft
1 3 inch driver step pulley to fit 1/2 inch motor shaft
1 20 inch driver belt

*From the Department of Bacteriology, School of Medicine, the University of Pennsylvania, Philadelphia.

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4 bolts for mounting shaft
4 bolts for mounting motor
2 three-way switches
1 male end for electric cord and a few feet of rubber-covered electrical extension cord
Miscellaneous pieces of lumber, nails, and screws for assembling cabinet.

The articles are all standard equipment and represent a total cost of less than $22.00. The assembled unit is shown in Figs. 1 and 2.

At the base and on the inside of each of the two side panels is nailed a 3½ inch strip. Resting upon these strips and fastened to the sides is the base for mounting the motor. On the underside of this base is mounted the ¾ inch shaft by means of the flat boxes. The motor is then mounted upon the base so as to give the proper tension on the belt. To the motor is attached an electrical extension cord by means of two three-way switches. A 1 inch hole is drilled in the front panel for the shaft, and the panel is secured in position by means of screws.

The purpose of a 3-way switch on each side of the washer is to enable the washer to be operated conveniently by either a left-handed or right-handed worker. The advantage of the tapered chuck is that the shaft may revolve in either direction without the danger of the chuck coming loose. The speed of the shaft should not be more than one-third that of the motor. Under ordinary conditions the motor runs at 1,725 r. p. m.; by means of the step-down pulleys the shaft revolves at 575 r. p. m. At this speed very little water is thrown from the brush, so we have not found it necessary to equip the washer with a shield to protect the worker. It requires only a few seconds to loosen the jaws of the chuck, insert a brush, and retighten the chuck. The compactness of the power washer enables it to be carried from one room to another or from one work table to another.

Brushes.—Satisfactory brushes, to be used by hand or with the power washer, for cleaning the usual laboratory glassware are not readily available on the market. The brushes which we now use for hand or machine work have been designed of a twisted-in-wire construction with the cooperation of the Fuller Brush Co., Industrial Division, Hartford, Conn., and have the following specifications (Fig. 3).

No. 1H706 for cleaning serologic tubes (10 mm. by 100 mm., outside diameter) by machine. Diameter ½ inch, brush length with tuft 3¼ inches, overall length 6⅜ inches. Black bristle. Wire handle straight for inserting into chuck.

No. 1H707* for cleaning serologic tubes by hand. Same specifications as for above brush 1H706, except that finger loop is left on end of handle so that the brush can be used by hand.

No. 1H708 for cleaning Wassermann tubes (13 mm. by 100 mm. outside diameter) by machine. Diameter ½ inch, brush length with tuft 3½ inches, overall length 6¼ inches. Black bristle. Wire handle straight for inserting into chuck.

*We have found it advantageous to have a few of the brushes made up with a loop on the end of the handle so that the brush can be used by hand. Occasionally it is desirable to brush a few tubes while the power washer is in use or a different sized brush is in the chuck.

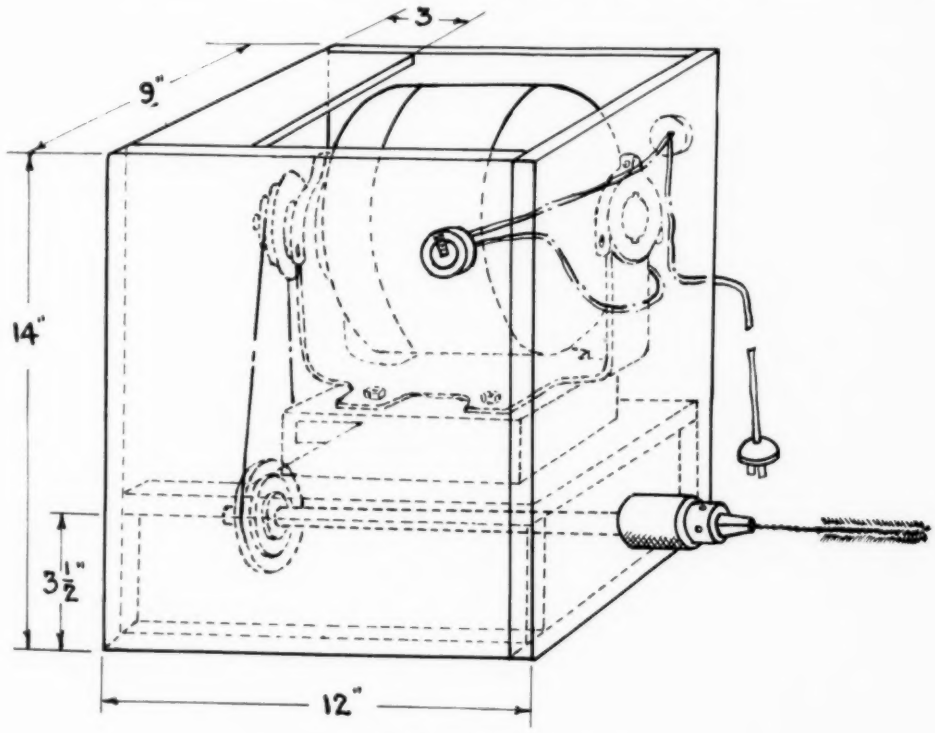

Fig. 1.—Electric test tube washer.

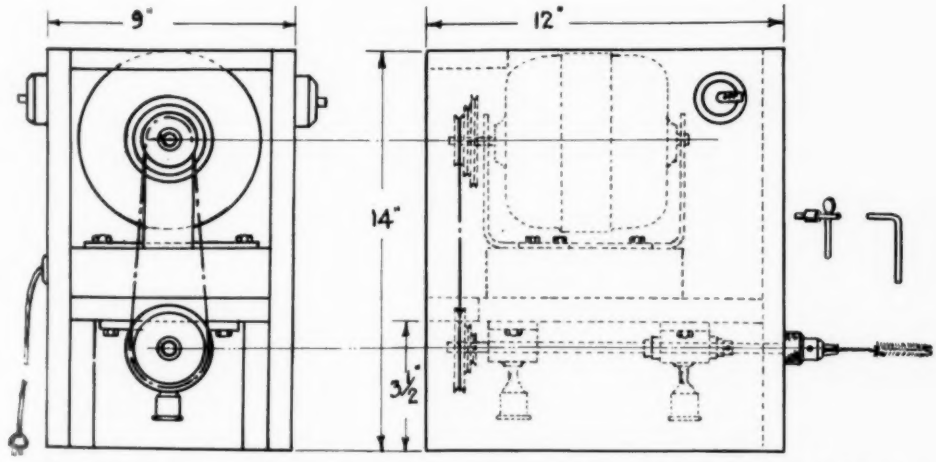

Fig. 2.—Electric test tube washer. *A*, Rear view; *B*, Side view, showing brush in place.

No. 1H709* for cleaning Wassermann tubes by hand. Same specifications as for above brush 1H708, except that finger loop is left on end of handle so that the brush can be used by hand.

No. 1H704 for cleaning ordinary test tubes (16 mm. by 150 mm. outside diameter) by machine. Diameter ⅝ inch, brush length with tuft 3⅛ inches, overall length 8½ inches. Black bristle. Wire handle straight for inserting into chuck.

*See footnote bottom page 212.

No. 1H705* for cleaning ordinary test tubes by hand. Same specifications as for above brush 1H704, except that finger loop is left on end of handle so that the brush can be used by hand.

No. 1H703 for cleaning large test tubes (25 mm. by 200 mm. outside diameter) and 50 c.c. centrifuge bottles by machine. Diameter 1 inch, brush length with tuft 3¼ inches, overall length 10½ inches. Black bristle. Wire handle straight for inserting into chuck.

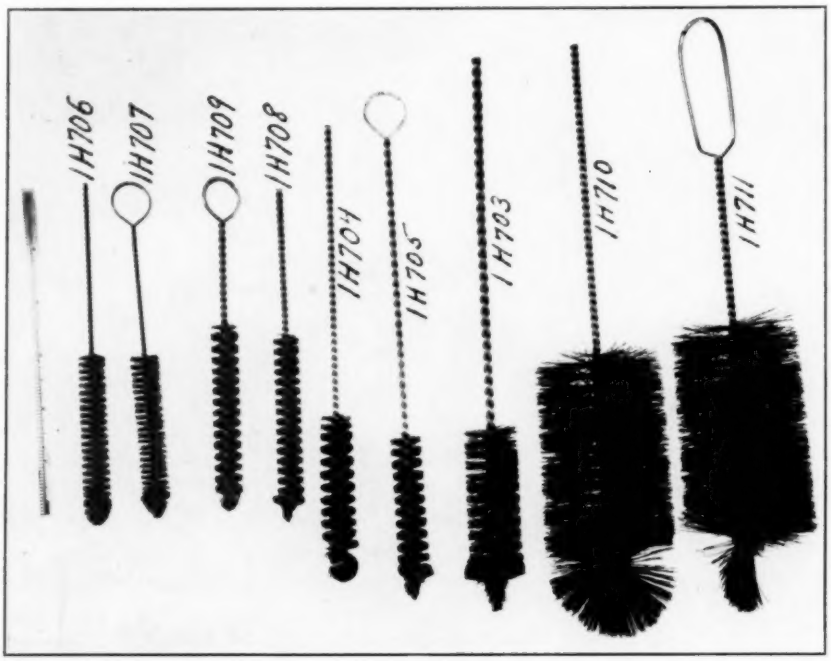

Fig. 3.—Brushes designed for machine or hand cleaning of laboratory glassware. For a description of the individual brushes see text.

No. 1H710 for cleaning 250 c.c. centrifuge bottles and 125 c.c. Erlenmeyer flasks by machine. Diameter 2⅜ inches, brush length with tuft 5 inches, overall length 11 inches. Black bristle. Wire handle straight for inserting into chuck.

No. 1H711* for cleaning 250 c.c. centrifuge bottles and 125 c.c. Erlenmeyer flasks by hand. Same specifications as for above brush 1H710, except that loop is left on end of handle so that the brush can be used by hand.

If it is necessary to clean glassware other than the above-mentioned standard sizes, satisfactory brushes can be designed as were these brushes.

The Cleaning Process.—Contaminated glassware is autoclaved before handling, the cotton stopper removed, and any fluid poured out while still warm. The cotton stoppers are removed from other glassware, and any liquid, if present, is poured out. The glassware is then completely submerged in dish pans of suitable size (we have found the oblong, white enamel baby baths most convenient), containing about half an ounce of Char-Lab washing powder† dis-

*See footnote bottom page 212.

†Obtainable from Eimer and Amend, New York, N. Y.

solved in about 3 gallons of warm water. The solution of washing powder is brought to a boil and boiled for about fifteen minutes. The solution is cooled or replaced with cool tap water until the glassware is cool enough to handle.

A brush of the proper size is placed in the chuck of the power washer, the chuck is tightened, and the brush set in motion by turning on the power washer. It is best for the cleaner to wear rubber gloves and also to wear over the rubber glove of the right hand an ordinary white canvas glove which serves two very good purposes: (1) as a wash cloth and (2) as added protection. A handful of tubes is picked up out of the wash water with the left hand, and without emptying the wash water out of the tubes, they are cleaned one by one on the revolving brush. A tube is grasped firmly by the right hand and shoved over the revolving brush as far as possible. There is practically no danger whatsoever of the end of the brush breaking the tube as the brushes are constructed with a very good tuft. By the time the tube has been shoved over the brush until the tuft comes in contact with the end of the tube, the inside of the tube is cleaned, and the tube may be withdrawn from the brush.

Usually the outside of the tubes is also soiled, as with wax pencil marks. These wax marks are very easily removed, if while the tube is still on the brush, the grip on the tube is released slightly so as to allow the tube to revolve against the canvas glove which is being worn over the rubber glove.

It is the practice of our cleaner to run the tube over the brush as far as possible, release the grip on the tube slightly so as to allow the tube to revolve against the gloved hand, hold the palm of the gloved hand against the end of the tube for a second, then run the gloved hand along the sides of the tube to the open end of the tube, tighten the grip on the tube, pull the tube off the brush and drop it into a vat of running warm tap water. The glassware after being well rinsed in the running warm tap water is given several rinsings in two vats of clean, distilled water.

Under no consideration should the hand be removed from a tube while it is revolving on the brush! The power washer should be stopped by turning off the switch, and the hand removed from the tube only after the tube has stopped revolving.

It has been our experience that the breakage of glassware with the power washer is less than when the glassware was cleaned by hand with the usual test tube brushes. By actual timing, glassware can be cleaned in one-fourth the time which was required for brushing by hand. The glassware is cleaned better, and is not fogged by the numerous repetitions of the cleaning process. Another economical feature is that the glassware does not have to be further cleaned by sulfuric acid-potassium dichromate solution.

SUMMARY

An inexpensive power washer is described which is far superior to the hand process for cleaning glassware.

Brushes for cleaning the usual laboratory glassware are described which are more economical and efficient than any heretofore obtained.

These innovations bring about great savings in time and materials.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

COCCIDIOIDAL GRANULOMA, Arrested Pulmonary, Cox, A. J., and Smith, C. E. Arch. Path. 27: 717, 1939.

Four cases of completely arrested coccidioidal granuloma of the lungs or of the bronchial lymph nodes in man are reported. In none of these were the granulomatous lesions a factor in the cause of death.

Similar arrested lesions could be produced at will by injecting small numbers of spherules of a virulent strain of the fungus coccidioides intravenously into white rats or guinea pigs. The minimum fatal inoculum for rats inoculated in this way was approximately 10,000 spherules.

In nearly all the arrested experimental lesions organisms remained viable for periods up to two and a half years. In a human case organisms were cultured fifteen years after they had been first demonstrated.

It is concluded that instances of arrested coccidioidal granuloma are more frequent than has heretofore been realized, that they probably outnumber the fatal cases of this disease, and that they are not restricted to the region of the San Joaquin Valley, Calif. The relationship of arrested coccidioidal granuloma to the benign clinical disease known as "Valley fever" is as yet unknown. Although the lesions present a histologic picture of inactivity, they may contain viable fungi for many years and constitute a possible source of dissemination.

BLOOD: Total Differential and Absolute Leukocyte Counts and Sedimentation Rates Determined for Healthy Persons Nineteen Years and Over, Osgood, E. E., Brownless, I. E., Osgood, M. W., Ellis, D. M., and Cohen, W. Arch. Int. Med. 64: 105, 1939.

The proportion of lymphocytes is higher and the proportion of neutrophile lobocytes (polymorphonuclears) is lower than the textbook figures. The probable explanation for the retention of erroneous figures in the textbooks is that these figures represent fairly closely the usual counts of patients sick enough to be attending an outpatient clinic or to be hospitalized, but who are not suffering from a generally recognized cause of marked leucocytosis. The dangers of using such persons as normal subjects are obvious.

In the age group 19 years and over, the leucocyte count is lower, the neutrophile percentage is higher, and the lymphocyte percentage is lower than the corresponding figures for younger persons. It is of interest that the absolute number of neutrophile lobocytes (polymorphonuclears) averages 4,000 per c.mm. for all ages from 4 to 30 years, except for the age group 8 to 14 years.

The sedimentation rates form a skew curve, with the greatest number of determinations falling in the lower ranges. It is probable that the rate of 15 mm. in forty-five minutes, which includes 80 per cent of the results, represents the strict upper limit of normal values, and that the higher rates are due to mild chronic infection in the tonsils, teeth, or sinuses not detectable in the routine physical examination.

There are no significant age or sex differences in the total, differential, or absolute leucocyte counts, or in the sedimentation rates in persons 19 to 30 years of age.

TISSUE: Differential Stain for Cell Types in the Pancreatic Islets, Gomori, G. Am. J. Path. **15**: 497, 1939.

Fixation: The thickness of pieces of tissue should not exceed 2 mm. in order to ensure rapid and complete penetration by the fixative. Aqueous fixatives, such as formalin, Bouin's, Zenker's, and Stieve's solution, or Zenker-formalin, may be used. The best fixative proved to be a modified Bouin's solution in which half the amount of acetic acid is replaced by sulfosalicylic acid (formalin 1 part, saturated picric acid solution 4 to 5 parts, acetic and sulfosalicylic acid 2.5 per cent each). For use, dilute this fixative with equal parts of distilled water. The staining of tissues fixed in any fluid can sometimes be greatly improved by refixing the sections before staining in the undiluted solution for twelve to twenty-four hours. The removal of mercury salts from tissues is done by the routine iodine method.

Embedding: Embed tissues in paraffin.

Oxidation: Deparaffinized (and refixed, if necessary) sections are treated for one minute with a solution containing about 0.3 per cent each of potassium permanganate and of sulfuric acid. The sections are rinsed in water and then decolorized with a 1 to 5 per cent solution of potassium metabisulfite. After decolorization the sections are thoroughly washed in water. Without oxidation the beta granules will not take the stain. For sections of pituitary, oxidation, though it will enhance the color contrast, is not strictly necessary.

Staining: Stain in a well-ripened solution of chromium hematoxylin for from fifteen minutes to one hour under microscopic control. The beta granules should be a deep blue, and the cytoplasm of the alpha cells should be unstained. In the pituitary the basophiles should be a deep blue, and the oxyphiles unstained. Any mucoid material is stained a deep blue. The staining solution is made up as follows:

Mix equal parts of a 1 per cent aqueous solution of hematoxylin and of a 5 per cent solution of chromium alum (potassium chromium sulfate). The brownish mixture is ripened by the addition of about 3.5 c.c. of a 5 per cent potassium dichromate solution plus a few drops of sulfuric acid to each 100 c.c. of the mixture. The process of ripening will take one or two days. The solution is ready for use as soon as it is a deep blue black with a purplish tinge. It should be filtered before us as a precipitate will form repeatedly on its surface. For some time the staining power and selectivity of the solution increase, but as the solution becomes older, the staining time has to be prolonged. Solutions that do not stain deeply enough in thirty-five to forty minutes are better discarded as they often do not stain with sufficient precision.

After staining in this chromium hematoxylin solution, rinse the sections in water and transfer to alcohol containing 1 per cent of hydrochloric acid. In this acid-alcohol solution the color of the section becomes a clearer blue. Rinse again and counterstain rather heavily with any of the routine red or orange acid dyes. Phloxine and ponceau de xylidine have been found to be especially suitable. The latter is used in a 0.5 per cent solution with 1 per cent of acetic acid. Rinse in water and differentiate in a 5 per cent solution of phosphotungstic acid until the acid dye is completely removed from the connective tissue. Only the strongly oxyphilic structures, such as erythrocytes, muscle fibers, oxyphiles, and alpha cells, remain stained. Carry sections through graded alcohols, clear in xylol, and mount in balsam.

TRICHINELLA SPIRALIS: Incidence of Infection in Man, Dogs and Cats in the New Orleans Area, Sawitz, W. Arch. Path. **28**: 11, 1939.

Examination of human diaphragms and pectoral muscles obtained in 400 routine unselected necropsies disclosed 24 cases of infection with *T. spiralis* in the New Orleans area, an incidence of 6 per cent. The compressor method detected 2 cases; the digestion method, 24. Of the 23 cases in which both the diaphragm and the pectoral muscle were available, the diaphragm was found infected in 20 cases, or 87 per cent, and the pectoral muscle in 13 cases, or 56.5 per cent. Surveys in which diaphragms only were examined would thus miss 13 per cent of the cases. The average number of larvae of *T. spiralis* found in the diaphragm was 0.35 per gm.; the average number in the pectoral muscle, 0.22 per gm. The diaphragm is,

therefore, not only qualitatively but also quantitatively the better tissue for examination. No history of clinical symptoms of trichinosis was found in any of the 24 cases. With increasing age the incidence of trichinella infection increased. The highest incidence was found in Negro females (6.66 per cent); the lowest, in white females (5.8 per cent).

The incidence of trichinella infections in 300 dogs in the New Orleans area was found to be 1.3 per cent; the incidence in 90 cats was found to be 10 per cent. The incidence in cats is considered to serve as an indicator of the endemicity of trichinella infection in an area.

TUBERCULIN TEST, Evaluation of the Patch, Vollmer, H., and Goldberger, E. W. Am. J. Dis. Child. **57**: 1272, 1939.

A comparative study of the tuberculin patch test (Lederle-Vollmer) and the Mantoux test has been carried out on 678 children.

Among 417 children admitted to the wards of the Mount Sinai Hospital, the reliability of the tuberculin patch test, as compared with the Mantoux test performed with 0.1 mg. of old tuberculin, was 100 per cent.

Two hundred and sixty-one tuberculous children of the Sea View Hospital showed 100 per cent conformity between the tuberculin patch test and the Mantoux test with first strength solution of purified protein derivative, or 0.01 to 1 mg. of old tuberculin. Only one child with positive anergy failed to react to the patch test as well as to the Mantoux test with first strength purified protein derivative. This child reacted positively to the Mantoux test with second strength purified protein derivative.

Consequently, it seems that the Mantoux test with 0.1 mg. of old tuberculin or less, or with first strength solution of purified protein derivative, can safely be replaced by the tuberculin patch test. The routine follows:

1. The tuberculin patch test (Lederle) is applied and read two days after removal of the patches. 2. Negative reactors to the patch test are retested with the Mantoux test with 1 mg. (0.1 c.c. of solution 1:100) of old tuberculin or second strength solution (0.005 mg.) of purified protein derivative. 3. Since higher concentrations of tuberculin occasionally cause pseudopositive reactions, both tests are repeated if there is any discrepancy. If the discrepancy remains, the Mantoux reaction is regarded as the deciding one.

This procedure is advocated for exact diagnosis in hospital wards. For general use in practice and for mass examinations the tuberculin patch test alone may suffice.

SULFANILAMIDE, Cyanosis From Use of, Bigler, J. A., and Werner, M. Am. J. Dis. Child. **57**: 1338, 1939.

From the available accumulated data it is evident that the cause and the mechanism of production of the cyanosis that so frequently occurs during sulfanilamide therapy are still not clear. While in some instances spectroscopic examination of the blood has revealed the presence of sulfhemoglobin and methemoglobin bands, in other instances it has given negative results. The cyanosis may be present in the presence or absence of nonfunctional hemoglobin. When nonfunctional hemoglobin is present, it has some, but not all, of the characteristics of methemoglobin. Further, the cyanosis does not seem to be dependent on the oxygen saturation of the arterial blood. Certainly, further studies of this problem are necessary for clarification.

THYROID GLAND, Histologic and Histochemical Structure of the Normal, Hertzler, A. E. Arch. Surg. **38**: 417, 1939.

A study of the thyroid glands of supposedly normal persons presents confusing variations. Many show changes parallel with those found in goiters from patients with goiter heart, that is, cardiac disease which disappears after removal of the goiter. Naturally these studies of the nongoitrous gland lead surgeons to ask themselves certain questions. What is the meaning of these changes in chemical reaction of the colloid in allegedly normal thyroids? No one knows, but it is known that these changes are found in goiters

from patients with "goiter hearts" and that in such a patient the heart recovers when the goiter is completely removed. So much is incontrovertible. This well-demonstrated clinical fact causes one to consider that possibly a degenerated thyroid gland, even though it has not acquired goitrous dimensions, may injure the heart. If this is true, the presence of a "goiter heart" demands the removal of the thyroid gland whether it is goitrous or not. First of all, one must recognize a "goiter heart" by clinical examination. It has been demonstrated to the author's satisfaction that, in the absence of a palpable goiter, if "goiter heart" is present, the removal of the thyroid, enlarged or not, rids the patient of the cardiac disturbance. These observations must be multiplied a thousandfold.

This presents the original question: How big must a thyroid gland be in order to become cardiotoxic? The corollary is, what changes in the thyroid gland must be present before it can be declared the cause of the cardiac symptoms? This is easily decided if one follows the aftercourse. If the patient recovers from the cardiac signs, one may conclude that the part removed was the cause. Multiply such an observation by thousands, and the conclusion is as accurate as one can hope to achieve. The patient, collectively speaking, is always right. If anatomic study does not corroborate clinical experience, one must extend the laboratory study. The surgeon should remember that when he sends a goiter to the laboratory, he does so to secure, not a diagnosis, but an opinion.

This is the problem before surgeons today. Since it is now known that total removal of the thyroid gland from the adult is a matter of indifference to the patient, it becomes merely a question of operative technique.

The tinctorial, as well as the histologic, study of the thyroid gland indicates the presence of certain changes which occur with advancing years. These changes parallel to some extent those with "goiter heart."

The physicians' knowledge of the relation of the nongoitrous gland to diseases of the heart is at present represented by a large question mark, but even this may presage an advance in understanding of the relationship between the nongoitrous and the goitrous gland.

The similarity of structure suggests that the effect of the thyroid gland is a matter of function, not of size. One may determine the nature of this function by removing the gland and observing the results.

ANEMIA, Exogenous Pernicious, Alsted, G. Am. J. M. Sc. **197**: 741, 1939.

A case of pernicious anemia is reported in a man of 43. It is shown that the development of anemia was due to deficiency of "extrinsic factor," continuous during seven to eight years. Treatment with "extrinsic factor" exclusively was followed by complete recovery and complete restoration of acid secretion in the stomach. The patient was controlled during seven months subsequent to the discontinuation of the specific treatment, and no relapse was observed.

The case is conceived as a case of exogenous pernicious anemia.

SYPHILITIC REAGIN, in Blood and Spinal Fluid, Wiener, A. S., and Derby, I. M. Arch. Dermat. & Syph. **39**: 999, 1939.

The concentrations of syphilitic reagin in serum and spinal fluid were compared in a series of 1,245 pairs of specimens by a quantitative flocculation technique. It was found that, with the method used, titers as high as 500 were attained in the blood serum, whereas the titer of the spinal fluid never exceeded 16. Moreover, the amount of reagin in any spinal fluid was practically always less than that of the corresponding serum. The bearing of these observations on the question of the site of origin of syphilitic reagin in the spinal fluid of neurosyphilitic patients is discussed.

SULFANILAMIDE, Observations Concerning the Absorption of, From the Large Intestine in Man, Turell, R., Marine, A. W. M., and Nerb, L. Brooklyn Hosp. J. **1**: 90, 1939.

From the experiments reported, it is evident that in man sulfanilamide administered by rectum in aqueous solution is absorbed from the large intestine and from the isolated rectum.

No changes were observed in the normal mucous membranes of the rectum and sigmoid following the introduction of sulfanilamide solution into the rectum.

It has been shown that the concentration of sulfanilamide in the blood, after administration of the drug by rectum, is sufficiently high to recommend this route of administration when sulfanilamide cannot be given by mouth.

"WINDOW PATCH" TEST, Guild, B. T. Arch. Dermat. & Syph. **39**: 807, 1939.

A description is given of a technique of patch testing which the author calls the "window" patch test. It is valuable for the following reasons:

1. It makes possible the frequent applications of buffered solutions to the test site and thus may be used to study the influence of alkalinity or acidity on the development of reactions of sensitivity or irritability of the skin.

2. It is useful for testing reactions of the skin to volatile substances, as repeated applications may be made beneath the glass window.

3. It provides constant visibility of the test site for both the patient and the physician; consequently, the development of serious reactions may be avoided.

4. The test site may be exposed to light; therefore, the method should be valuable in studying certain dermatoses caused by substances, the cutaneous reactions to which may depend on exposure to light.

The method follows: A microscope slide is cut with a glass cutter into three equal sections, each 1 inch (2.5 cm.) square, and the edges are well smoothed by means of an emery wheel or a suitable file. A strip of adhesive plaster, ¾ inch (1.9 cm.) wide and about 2½ inches (6.2 cm.) long, is applied to each of three sides of the glass square, so as to secure it to the skin and at the same time allow a portion of the glass to remain uncovered for the purpose of observation. The substance to be used in the test is then placed directly on the skin and covered by the central clear portion of the glass square. The ends and free edges of the strips of adhesive tape are then stuck tightly to the skin. The device is commonly referred to in my laboratory as the "open" patch, because the upper edge of the glass has been left free, making it possible to insert fluids beneath the glass.

PNEUMONIA, VIRUS of Infants Secondary to Epidemic Infections, Goodpasture, E. W., Auerbach, S. H., Swanson, H. S., and Cotter, E. F. Am. J. Dis. Child. **57**: 997, 1939.

Five cases of a hitherto undescribed virus infection of the lungs of infants, especially following measles and whooping cough, have been observed and recorded.

The virus invasion usually appeared to be secondary and tended to pave the way for bacterial infection of the lungs.

The presence of the virus was indicated by the occurrence of nuclear inclusions in epithelial cells of the trachea and bronchi and their mucous glands, and of the alveolar epithelium. Rapid necrosis of the affected cells occurred, resulting in ulceration of surfaces.

The virus appeared to be different from that of herpes simplex and from the agent of the so-called inclusion disease of infants.

Experimental inoculation of infected lung tissue into rabbits, mice, opossums, chicken embryos, and a *Macacus rhesus* monkey, failed to establish the infection.

REVIEWS

Books and Monographs for Review should be sent direct to the Editor, Dr. Warren T. Vaughan, Professional Building, Richmond, Va.

Rural Medicine*

AMONG the more popular "indoor sports" of the moment appears to be the preparation of data—statistical or otherwise—concerned with availability or nonavailability of medical care; it is somewhat unfortunate that some, at least, of these bear evidence of having sprung from bias or of having been conceived from preformed opinion.

Alive to this problem and thoroughly familiar with rural environment, the staff of the Mary Imogene Bassett Hospital at Cooperstown, New York, decided to analyze their own experience and, for this purpose, called a conference to consider the results obtained.

The facts elicited are presented in this volume and are discussed by outstanding workers interested in this phase of medical practice.

The volume is divided into four main sections representing the subjects discussed at the four meetings: Rural Morbidity; Health Department Programs and School Health Programs in Rural Areas; Postgraduate Medical Education in Rural Areas; and Economics of Rural Medicine.

A bibliography of rural medicine concludes the book.

This is perhaps one of the more important of the recent contributions to this field, if not, indeed, among the most important, principally, perhaps, because it sets out to *prove* nothing. The object was to *find out* the true state of affairs in a representative rural community and from this, if possible, to draw such inferences and conclusions as were legitimate. The particular value of this study lies, first, in its *factual* character and, second, in its broad approach.

It is a volume which deserves careful reading for its stimulating effect and might well be studied by both the radical and the conservative in the ranks of the medical profession. The sociogist, economist, and all who are busy in this matter may well read and study it with profit.

Crystalline Enzymes†

IN THIS monograph the author presents the results of a series of investigations on the proteolytic enzymes and upon the isolation and chemistry of bacteriophage. While the latter has not yet been crystallized nor, indeed, yet shown to be definitely an enzyme, the results of experiments with it are similar to those obtained with enzymes and so presented with them. Six chapters are given to the discussion of enzymes and the results obtained with them in crystallized form; the seventh chapter discusses bacteriophage. An appendix gives the methods used in full detail. There is a full bibliography and an index.

This book should be of great interest to the biochemist and physiologist.

Advances in the Therapeutics of Antimony‡

WITH the use of antimony taking on renewed importance in the treatment of a variety of diseases of man and beast, this most excellent résumé of the present status of the pharmacology and therapeutics of antimony compounds should be most welcome. As a

*Rural Medicine. Proceedings of the Conference Held at Cooperstown, New York. Cloth, 268 pages, $3.50. Charles C. Thomas, Springfield, Ill.

†Crystalline Enzymes, the Chemistry of Pepsin, Trypsin and Bacteriophage. By John H. Northrop, Member of Rockefeller Institute for Medical Research. Cloth, 176 pages, 48 figures, $3.00. Columbia University Press, New York.

‡Advances in the Therapeutics of Antimony. By Hans Schmidt, Ph.D., and F. M. Peter, M.D. With a Preface by Philip Manson-Bahr, M.D. Paper, 257 pages, 10 diagrams. Georg Thieme, Leipzig.

preface Dr. Philip Manson-Bahr has written most interestingly of the history of antimony. The authors then discuss the clinical experience with antimony compounds in human and veterinary medicine, citing in a very attractive and novel manner the world literature. The synthesis of various preparations is discussed with proper bibliographic references. Then there are some thirty pages devoted to tests in which antimony preparations were used on animals infected experimentally. The authors close with a thorough description of the pharmacology of antimony and with a discussion of some of the newer preparations available.

The reviewer feels that this is a book that should be read by all those interested in the therapeutic application of antimony to infectious diseases and those interested in chemotherapy. It is an excellent book.—*H. B. Haag.*

Manual of Urology*

THE avowed purpose of this book is to present "the fundamentals of urology in such a way that they can be easily grasped by the student approaching this subject."

This second edition, extensively revised and rewritten, will without doubt maintain the reputation achieved by its predecessor, characterized as "the most compact and yet basically complete book on urology available."

Professor Le Compte, in addition to being master of his subject, possesses the happy knack of being able to present it in a clear-cut and assimilable manner.

A good book for the physician as well as the student.

Textbook of Medicine†

THIS, the fourth edition, of a well-known English reference text, has been thoroughly revised to include the advances made since the appearance of the preceding edition in 1936.

Though necessarily in covering the whole field of medicine, the articles are of various lengths and in the main brief, they are nevertheless comprehensive and cover the subjects systematically as well as concisely.

A good "refresher" reference text.

Pye's Surgical Handicraft‡

AS THE editor of this book says in his preface, when a medical book, and especially a book concerned with so flexible a subject as surgery, retains its place for fifty-four years, it can be safely assumed that it is a good book, a useful book, and one which may be relied upon.

The eleventh edition of this standard work worthily carries on the reputation and tradition of its predecessors.

The house surgeon and surgical intern, indeed the intern at large, will find this volume of inestimable value. The physician at large may well and profitably add it to his shelf of working references.

Altogether it is an excellent book, and in an emergency is well worth many times its price.

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*Manual of Urology. By R. M. Le Compte, M.D., F.A.C.S., Professor of Urology, Georgetown University Medical Department. Cloth, ed. 2, 295 pages, 54 figures, $4.00. Williams & Wilkins Co., Baltimore, Md.

†Textbook of Medicine. Edited by J. J. Conybeare, M.C., and D. M. Oxon, F.R.C.P., Physician to Guy's Hospital, London. Cloth, ed. 4, 1112 pages, 26 plates, 24 figures. Williams & Wilkins Co., Baltimore, Md.

‡Pye's Surgical Handicraft. A Manual of Surgical Manipulations, Minor Surgery, and Other Matters Concerned with the Work of the House Surgeons and Surgical Dressers. Edited by Hamilton Bailey, F.R.C.S., Surgeon Royal Northern Hospital, London; Surgeon and Urologist, Essex County Council; External Examiner in Surgery, University of Bristol. Cloth, ed. 11, 512 pages, 375 illustrations, $6.00. Williams & Wilkins Co., Baltimore, Md.

Peripheral Vascular Disease*

THE increasing popularity of the monograph is a logical aftermath of the constant and varied changes in the understanding both of disease in general and of diseases in particular. For, devoted to a single subject, the monograph is free to discuss it comprehensively and even exhaustingly.

In this book Collens and Wilensky have made a notable contribution to the study of peripheral vascular disease, a subject of great interest and increasing importance, not only to the specialist but also to the practitioner at large by whom the vast majority of such cases are first seen.

The book has two main parts: the first, in the authors' words, "devoted to anatomy, pathological physiology, symptoms, signs, methods of examination, diagnosis and differential diagnosis of vascular disorders"; the second, "a description, summary and evaluation of every known method of treatment, the application of the basic laws of physiology to treatment, the indications for the methods of treating each individual disease and the eventual prognosis." These objectives are fully attained in the book.

This book is not a theoretical, abstract, or superscientific discussion, but a clear, sane, and usable presentation based not only upon a thorough familiarity with the pertinent literature but also upon a comprehensive practical clinical experience. The book is well illustrated.

It is a book useful to the specialist, but perhaps even more valuable to the physician in general, and as such can be highly commended.

The Harvey Lectures†

THE thirty-fourth of a series of lectures, delivered under the auspices of the Harvey Society of New York, contains the following addresses: Some Aspects of the Intermediary Metabolism of the Steroid Hormones (Guy F. Marrian); The Significance of the Albumin Fraction of the Serum (Ashley Weech); Heat Loss From the Human Body (Eugene F. DuBois); Proteins as Chemical Substances and as Biological Components (E. J. Cohn); Observations on the Pathology of Rickets (Edwards A. Park); Distribution of Enzymes in Tissue and Cells (K. Linderstrøm-Lang); Genic and Hormonal Factors in Some Biological Processes (C. H. Danforth); and Biological Oxidation and Vitamins (Albert Szent-Gyorgyi).

The authors are all of international repute, and their lectures represent the outstanding advances in their respective fields.

Sclerosing Therapy‡

MUCH of the discussion of sclerosing therapy has arisen from failure to appreciate its limitations and contraindications as well as from attempts to utilize it without a thorough comprehension of its difficulties and niceties of technique.

In this book four of the outstanding exponents of this method discuss its applications, each in the particular field in which he has had extensive experience.

The Injection Treatment of Hernia is fully expounded by A. F. Bratrud, assistant professor of Clinical Surgery and Director of Clinic for Ambulant Treatment of Hernia, University of Minnesota. George F. Hock, Associate Attending Urologist, St. Luke's Hos-

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*Peripheral Vascular Disease: Diagnosis and Treatment. By William S. Collens, B.S., M.D., Metabolist, Chief of Clinic for Peripheral Vascular Disease, Chief of Diabetic Clinic, Israel Zion Hospital, Brooklyn; and Nathan R. Wilensky, M.D., Assistant in Clinic for Peripheral Vascular Disease, Assistant in Diabetic Clinic, Israel Zion Hospital. Cloth, 243 pages, 77 illustrations, 3 color plates, $4.50. Charles C. Thomas, Springfield, Ill.

†The Harvey Lectures, 1938-1939. Series XXXIV. Cloth, 279 pages, illustrated. $4.00. Williams & Wilkins Co., Baltimore, Md.

‡Sclerosing Therapy. The Treatment of Varicose Veins, Hemorrhoids, Hernia and Hydrocele by Injection. Edited by Frank C. Yeomans, M.D., F.A.C.S., Professor of Proctology and Attending Surgeon, New York Polyclinic Medical School and Hospital. Cloth, 336 pages, 185 illustrations, $6.00. Williams & Wilkins Co., Baltimore, Md.

pital, New York, discusses *The Injection Treatment of Hydrocele*, while *The Injection Treatment of Varicose Veins* is covered by Harold J. Shelley, Assistant Surgeon, St. Luke's Hospital, New York, and *The Injection Treatment of Hemorrhoids* by F. C. Yeomans.

This book is exceptionally practical in its aspect and detailed and precise in its presentation. It is an excellent book for those interested in this subject.

You Can't Eat That*

A GENERAL cook book for those with allergy. Part I describes allergy and the allergic type of reaction. The historical review of the early experimental work leading up to present-day clinical allergy is very well done, somewhat in the de Kruifian manner.

Part III, by far the largest section, contains recipes. These are not merely the wheat, egg, and milk avoidance recipes so familiar to those dealing with allergic persons. Instead, they cover the field of cookery for allergics in a comprehensive manner, stressing the need for tasty and palatable dishes to replace others which are usually so much regretted by those who must avoid them.

The third section contains general information on foods. It has Tuft's list of ingredients of nationally advertised food preparations, to which many additions have been made.

This volume should be of real service to those who must practice allergic dietary avoidances and who are fortunate enough to have someone in the kitchen willing to go to the trouble of trying new things.

Recent Advances in Physiology†

THIS, the sixth edition of a justly popular review series, has been thoroughly revised to bring the text in line with the advances in this field. Among the entirely new additions are discussions of the physiology of bone, the Carrel-Lindbergh perfusion apparatus, problems of carbohydrate metabolism, and cortical control of muscular movements. A useful book for those desiring a succinct but thorough review.

Recent Advances in Hematology‡

SO extensively revised as to be practically rewritten, with four additional color plates and sixteen added text illustrations, this new edition of Piney will, without doubt, maintain and enhance the repute achieved by its predecessors.

An excellent, comprehensive, and authoritative review of an important subject.

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*You Can't Eat That. A manual and recipe book for those who suffer either actually or mildly (and perhaps unconsciously) from food allergy. By Helen Morgan. Foreword by Dr. Walter C. Alvarez, of the Mayo Clinic. Harcourt, Brace and Co., New York, 1939.

†Recent Advances in Physiology. By C. Lovatt Evans. Revised by W. H. Newton, M.D., M.Sc., Reader in Physiology, University of London, University College. Cloth, ed. 6, 490 pages, 109 illustrations, $5.00. P. Blakiston's Son & Co., Philadelphia, Pa.

‡Recent Advances in Hematology. A. Piney, M.D., Ch.B., M.R.C.P., Assistant Physician, St. Mary's Hospital for Women and Children, London. Cloth, ed. 4, 312 pages, 34 illustrations, 8 color plates, $5.00. P. Blakiston's Son & Co., Inc., Philadelphia, Pa.